Science Library

MAR 19 1946

HEREDITAS

GENETISKT ARKIV

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ROBERT LARSSON

BAND XXVI HÄFT. 3—4

LUND 1940, BERLINGSKA BOKTRYCKERIET

HEREDITAS,

a periodical devoted to the publication of original research in heredity, is published by the MENDELIAN SOCIETY at Lund, Sweden. All contributions are written in English, German or French, illustrated, when necessary, with adequate text figures and plates. Each volume contains 400—500 pages, issued in four numbers.

Subscriptions should be sent to the Editor. Price *Twenty-five (25) Swedish Kronor per volume.* Post free. (The price of Vols. I—X is 10 Swedish Kronor per volume, of Vols. XI—XXIII, 15 Sw. Kr., and of Vols. XXIV—XXV, 20 Sw. Kr.)

ROBERT LARSSON
Editor of »Hereditas»
3 Adelgatan, LUND, SWEDEN.

REDAKTIONSKOMMITTÉ

PROFESSOR, FIL. DR. *ARNE MÜNTZING*
PROFESSOR, MED. DR. *HERMAN LUNDBORG*
PROFESSOR, FIL. DR. *HERIBERT NILSSON*
FIL. DR. *GUSTAV THULIN*

TWO EXTREME X-RAY MUTATIONS OF MORPHOLOGICAL INTEREST

BY *ÅKE GUSTAFSSON* AND *EWERT ÅBERG*
INSTITUTE OF GENETICS, SVALÖF, SWEDEN AND INSTITUTE OF PLANT HUSBANDRY, UPPSALA, SWEDEN

AMONG a series of X-ray mutations in barley, obtained in the experiments of recent years, the first-mentioned author found two types, which are of interest from a morphological and phylogenetic point of view. According to the latter author, working on the taxonomy and phylogeny of *Hordeum*, these types are not previously met with in the literature.

Both mutations have been obtained from a pure line, Golden barley (»Gullkorn»), isolated at Svalöf already before 1900. Golden barley is a typical *Hordeum distichum nutans* type, of great importance to the Scandinavian barley breeding. The first mutation (the two-flower type) arose in the X_2-generation in 1937 from seeds irradiated very intensely in the spring of 1936. The X_1-generation showed a germination capacity of 31 %. The second mutation (with lemma-like glumes) arose in the offspring of a specially treated seed-series. 645 seeds were desiccated above conc. H_2SO_4 and then X-rayed. In the roots of some germinating seeds the frequency of chromosome disturbances was examined and, in fact, most cells in division contained fragments and two-centromere formations. The germination capacity was 22 % and only 7,7 % fertile plants were formed. The sterility in the X_1-generation was high, and the mother-plant of this mutation, which appeared in the X_2-generation, showed 66 % sterility.

The two-flower mutation. — Within each ordinary lemma two flowers are formed. This type is completely sterile and segregates as a recessive. The heterozygote cannot be distinguished from the dominant Golden barley-type. The segregation is in the ratio 3 : 1. In 1938 the actual segregation was 413 : 124 (D/m approximately equal to 1). The cause of the sterility is unknown. — The long-awned primary lemma is normal. The barbs of this lemma are less numerous and less pronounced than in Golden barley (3—4 per nerve). The spikelets of the middle-row contain two flowers, the lemmas of which have 4—7 barbs each, thus in closer agreement with the mother line. The awns

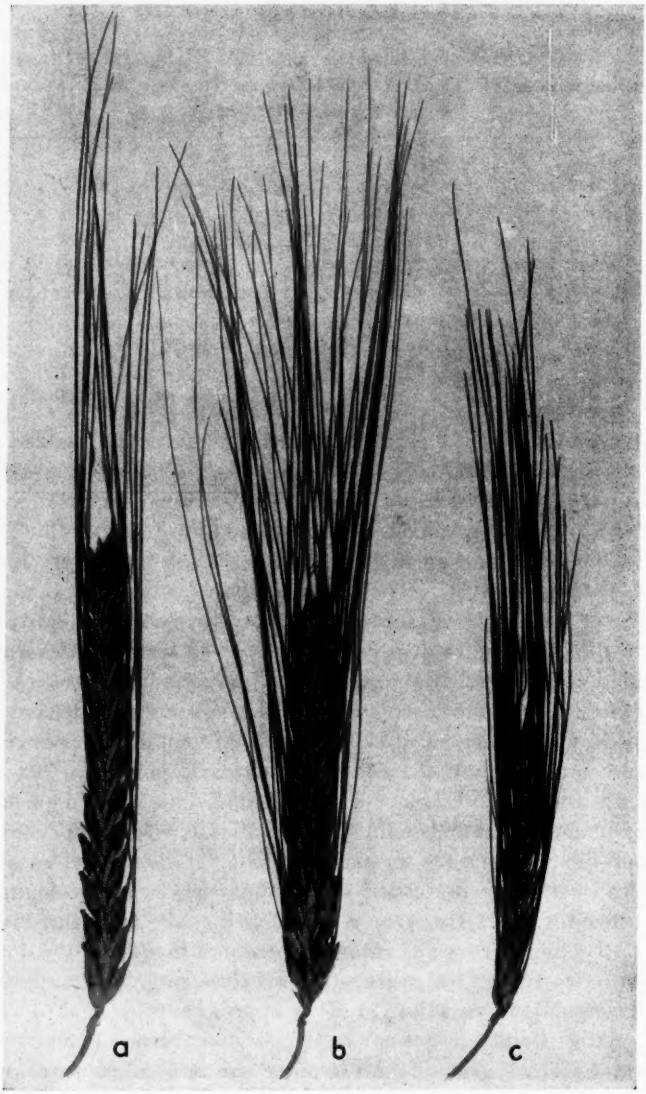


Fig. 1 *a*: Ear of Golden barley, *b*: of a mutation with lemma-like glumes, and *c*: of a mutation with two flowers within the primary (ordinary) lemma. — 1/1.

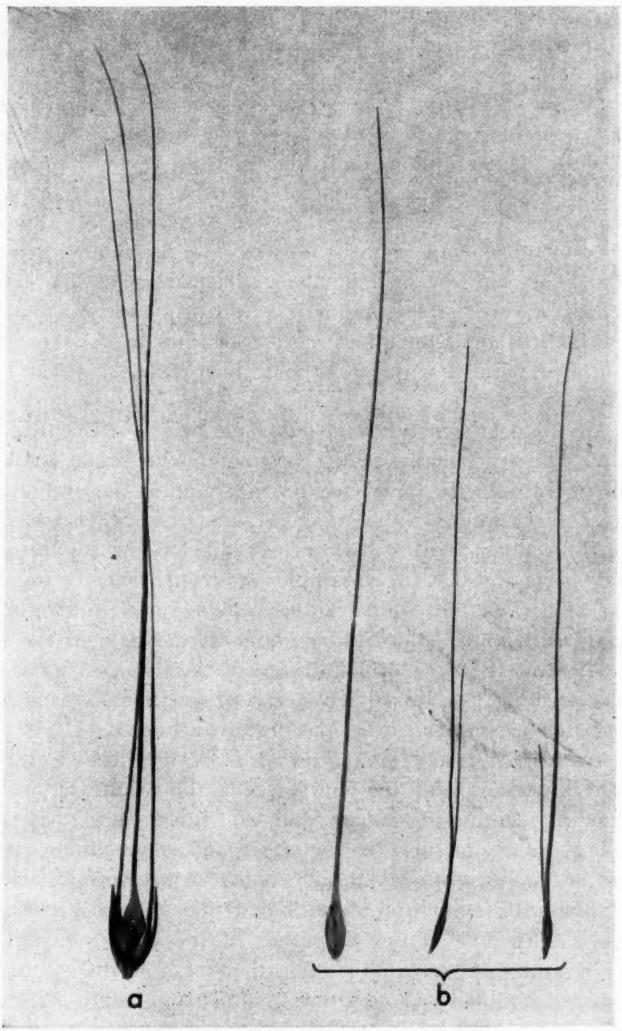


Fig. 2 *a*: Flower with lemma-like glumes and three long awns. *b*: To the left: the primary or ordinary lemma, to the right: the two individual flowers within such a primary lemma, each flower with one long and one short awn. — $^1/_1$.

of the secondary lemmas are fairly fine, about $^3/_4$ of the length of the awns on the primary lemmas. The paleæ have short awns about 2 cm.

in length. The barbs of the awns from all lemmas and paleæ agree in appearance with those in Golden barley. The rachillas are normal with long hairs.

A mutation with lemma-like glumes. — Due to its complete fertility and constancy this type is of direct breeding interest. Seeds are unusually large. Outer glumes look like lemmas, each of them having an awn of the same length as that of the lemma. (In Golden barley the glumes are linear with short awns.) The size of glumes and lemmas is identical. In contrast to the case in Golden barley the outer glumes are entirely without hairs. Barbs are missing. Barbs of the lemmas, however, are as numerous and pronounced as in Golden barley. All three awns have barbs. The lemmas in the side-rows are either blunt or pointed with or without short awns of about $1\,^1/_2$ cm. in length.

The anatomical structure and ontogenic origin of the two flowers within the primary lemmas in the first-mentioned mutation is so far unknown to us, and we have seen no taxonomical descriptions in the literature corresponding to this type. SCHIEMANN (1921) described the anomalous occurrence of two-flowered spikelets in the progeny of a special barley-cross. In a sample of *Hordeum vulgare* L. var. *pallidum* SÉR., VAVILOV found an unknown variety, differing from the normal in having a third outer glume at the base of the ear and small leaflets at the base of middle grains in the ear (VAVILOV and BUKINICH, 1929). This variety, named *afghanicum* VAV., is however only superficially similar to the one described here.

No cultivated variety or species of barley hitherto known from Europe or America shows the characters of the second mutation with regard to the lemma-like outer glumes. BOSE (1931) described a spontaneous variety of Pusa barley with broad outer glumes possessing awns and in 1937 he wrote about the heredity of this character. Whether the mutation with lemma-like glumes is identical to the variety studied by BOSE with regard to the properties of the glumes is difficult to tell. This mutation seems to indicate that the differentiation of outer glumes and lemmas has not become completely stabile. The similarity of this mutation to a variety of *Hordeum spontaneum* C. KOCH, named *ischnatherum* COSSON, is obvious, if the properties of the lemmas in the side-rows are taken into account. In his paper of 1908, KÖRNICKE has directed the attention to this feature of var. *ischnatherum*. The lemmas of the side-flowers in *H. spontaneum* are blunt as in cultivated *H. distichum* L. In the variety mentioned they were

either pointed or short and thin-awned. SCHWEINFURTH (1908) was of the same opinion. SCHULZ (1912) does not agree with KÖRNICKE and regards *ischnatherum* as a step forward from *H. spontaneum* to the six-rowed barleys. According to him, *H. spontaneum* var. *ischnatherum* has not only pointed or short-awned lemmas, but these can even be blunt. The X-ray mutation described above shows that pointed and short-awned types may arise from normally blunt varieties. That might be the cause of the origin of such variations in nature.

These mutations are of interest from a plant-breeding point of view. They show that it is possible to produce mutations outside the normal variability of cultivated and high-yielding plants. Especially the second mutation, which is completely fertile and viable, may be mentioned. Seeds are larger than in Golden barley, presumably due to an increased assimilation around the flowers. In a paper to be published shortly, one of the authors will describe a series of viable mutations produced after special treatments by X-raying. They fall, however, within the normal variation sphere of the cultivated barley. Planned viability and production tests will show whether these viable mutations are inferior, equal or, in some cases, even superior to the mother-line.

LITERATURE CITED.

1. BOSE, R. D. 1931. Studies in Indian barleys. I. Classification of types isolated at Pusa. — Indian Journ. agric. Sci. Vol. I.
2. — 1937. Studies in Indian barleys. IV. The inheritance of some anatomical characters responsible for lodging and of some ear-head characters in an interspecific cross between two Pusa barleys. — Indian Journ. agric. Sci. Vol. VII.
3. KÖRNICKE, FR. 1908. Die Entstehung und das Verhalten neuer Getreidevarietäten. — Arch f. Biontologie 2.
4. SCHIEMANN, E. 1921. Genetische Studien an Gerste. II. Zur Genetik der breitklappigen Gersten. — Zschr. f. ind. Abst.- u. Vererb.-lehre. Bd. 27.
5. SCHULZ, A. 1912. Die Geschichte der Saatgerste. — Zschr. f. Naturwiss. 83.
6. SCHWEINFURTH, A. 1908. Über die von A. AARONSOHN ausgeführten Nachforschungen nach dem wilden Emmer (*Triticum dicoccoides* KCKE). — Ber. deutsch. bot. Ges. 26 a.
7. VAVILOV, N. I. and BUKINICH, D. D. 1929. Agricultural Afghanistan. — Leningrad.

THE EFFECT OF ACENAPHTHENE AND COLCHICINE ON MITOSIS OF ALLIUM AND COLCHICUM

BY *ALBERT LEVAN*
CYTO-GENETIC LABORATORY, SVALÖF

THE effect of colchicine on the chromosome mechanism is of a distinctly different nature from the pathological phenomena caused by most other poisonous substances. The most characteristic feature in the action of colchicine is perhaps its extremely keen selectivity. While most other poisons bring about mitotic disturbances in general, colchicine affects only the spindle apparatus. Other vital processes of the chromosomes seem to continue normally, even during colchicine treatments of considerable length. In view of this fact it seems necessary to assume that the colchicine molecule has a specific ability to act directly upon the molecules which build up the spindle organs, the centrosomes and centromeres, while other physical and chemical agents cause more or less complex disturbances of mitosis. In order to stress this point I introduced the term c-mitosis to denote the abnormality of mitosis caused by colchicine.

It soon turned out that other substances as well as colchicine were able to cause c-mitosis. Thus SCHMUCK (1938), KOSTOFF (1938 a) and NAVASHIN (1938) reported that they had found another substance, acenaphthene, which had a similar effect to that of colchicine. And later on SCHMUCK and KOSTOFF (1939) and SIMONET and collaborators (for instance, SIMONET and GUINOCHET, 1939) directed their attention to several cyclic halogene derivatives with similar action. NEBEL (1938), however, failed to get any effect of acenaphthene in his material, staminal hairs of *Tradescantia*. And BLAKESLEE (1939) states that acenaphthene »seems relatively ineffective in inducing 4n plants in the species with which we have tested it» (p. 163).

If the effect of colchicine is due to a very specialized reaction, a temporary poisoning or inactivation of the spindle organs, it is *a priori* surprising that these relatively simple benzole and naphthalene derivatives are able to produce the same effect as the very complicated colchicine molecule. It therefore seems appropriate to make a critical comparison of the action of colchicine and these other substances on a

cytologically suitable material. The present paper deals with the effect of acenaphthene on the root chromosomes of the same *Allium fistulosum* clone, the reaction of which to colchicine was studied earlier (LEVAN, 1938). Its normal cytological conditions have been controlled for several years. In order to elucidate the problem also from another angle, the effect of colchicine and acenaphthene on *Colchicum* is described afterwards. *Colchicum* should for natural reasons be immune to colchicine. And BLAKESLEE (l. c.) met with a negative result of the macroscopical c-tumour reaction in *Colchicum*: »It is like the snake which is immune to its own venom» (p. 163). If *Colchicum*, in spite of its immunity to colchicine, is affected by acenaphthene the possibility must be considered as to whether two different processes necessary for normal spindle function are present, each of which may be affected separately, one by colchicine and the other by acenaphthene.

Owing to the insolubility of acenaphthene in water, the treatments of root tips with acenaphthene must be performed in a somewhat different way from the colchicine treatments. The smaller *Allium* bulbs were treated with acenaphthene by wrapping the roots in moist filter-paper and putting them in petri dishes, in which small amounts (about 1 g) of acenaphthene were placed. The larger *Colchicum* bulbs were placed in an acenaphthene atmosphere under a glass-bell. It was found that a positive effect could be obtained by placing the acenaphthene crystals on small watch-glasses at a short distance from the roots. Thus the vapour of the substance is effective. In order to obtain the maximal effect quickly, the best method, however, is to dust the roots directly with plenty of acenaphthene and then wrap them in moist filter-paper. This very strong treatment was not lethal as a rule, either in *Allium* or in *Colchicum*. Since the course of the changes in the mitoses were more regular following such maximal dosages, most of the experiments described below refer to such treatments.

My thanks are due to Dr. O. HAGERUP, Copenhagen, for providing me with *Colchicum* material, and to Miss M. PALM for valuable technical assistance.

I. ACENAPHTHENE EXPERIMENTS WITH ALLIUM.

A clear difference in action between colchicine and acenaphthene is observed in the very first appearance of the disturbances. As soon as the concentration of the colchicine solution tested is increased above the threshold value, the effect sets in with remarkable suddenness and com-

pleteness. In fact, the effect is maximal already a few minutes after the beginning of the treatment, and normal mitoses are entirely absent from the tissue. Acenaphthene acts decidedly more slowly, its initial period from the first visible action until full effect lasts for several days. Even after a treatment of 5 to 7 days plenty of normal anaphases may be found in the tissue and only after the longest treatments in my experiments was the tissue completely devoid of normal mitoses. On account of this extension of the initial period, we have a splendid opportunity here of studying the induced changes step by step in a manner impossible after colchicine treatments.

After a treatment of 4 to 24 hours most mitoses take place normally. A number of quite normal anaphases are seen exceedingly well in the longitudinal sections. Still the first signs of abnormalities are already present. The equatorial plates have not always developed quite regularly. One or two chromosomes may be located outside the plate, or the whole plate may be changed into a half-spherical shape, indicating that one of the centrosomes has been put out of function.

During the next period, a treatment of 1 to 4 days, more conspicuous changes of the spindle appear. These changes almost always begin in the exterior part of the spindle, in the centrosomes. Thus the congression of the chromosomes into the equatorial plate becomes more and more deficient. And when no trace of an equatorial plate can be detected any longer and the chromosomes, after the disappearance of the nuclear membrane, are scattered evenly over the entire cell, the centromeric part of the spindle still behaves normally: the centromeres divide and the half-centromeres repel each other. In other words, the interior part of the spindle still continues to function. During this breakdown of the exterior spindle there very frequently occur all kinds of abnormal spindles, such as mono-, tri- and multipolar spindles, resulting in the origin of a varying number of nuclei in each cell. These nuclei may often be separated by cell walls.

At the same time as this inactivation of the centrosomic spindle takes place, the first signs of an abnormal behaviour also of the centromeres can be observed. Often only one or two chromosomes of one cell begin to behave abnormally. Fig. 2 *a* pictures such an extreme case, where just the satellited chromosome has been affected, while the rest of the chromosomes form a normal bipolar anaphase. The first visible effect of acenaphthene on the centromeres is, in the same manner as after colchicine, a delay in the division of the centromeres. The relational spiral is completely or partly uncoiled before the division

of the centromeres (Fig. 1 *a*—*g*). The very typical cross-shaped c-pairs are formed in more advanced stages of the c-mitosis (Fig. 1 *h*—*i*). They are identical in appearance to the colchicine-induced c-pairs.

That the inactivation of the centromeres occurs gradually is de-

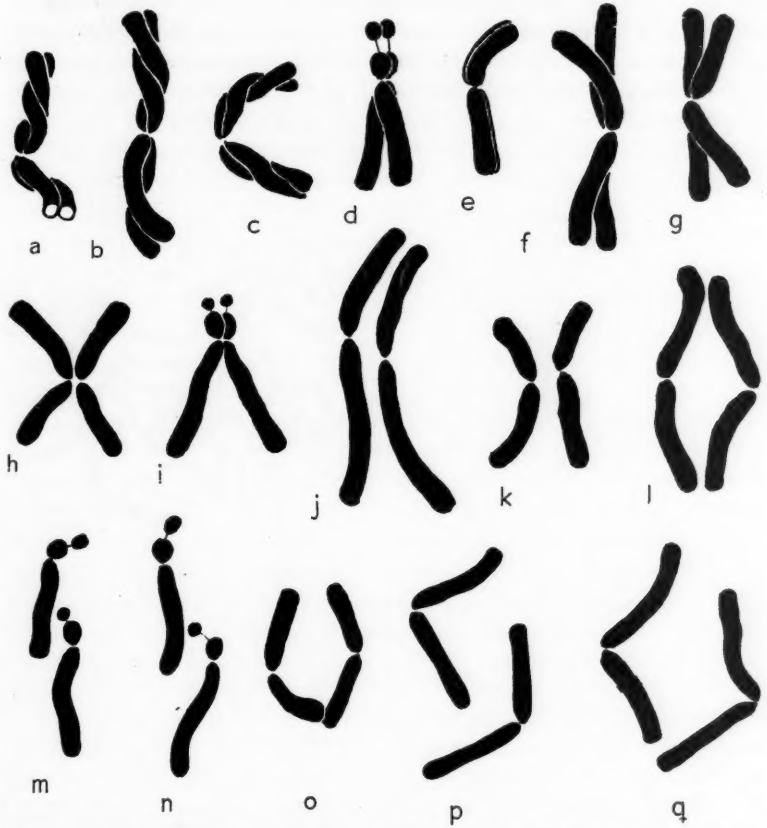


Fig. 1. *Allium fistulosum*, the development of the c-pairs after acenaphthene treatment, *a*—*g*: the uncoiling of the relational spiral, *h*—*i*: the cross-shaped c-pairs, *j*—*k*: the end stage of the c-pair formation, *l*—*q*: an earlier stage, where the repulsion force of the half-centromeres is still functioning. — × 3900.

monstrated, I think, by the following very typical phenomenon. Even when the division of the centromeres is delayed considerably the daughter centromeres nevertheless effect a repulsion on each other, when they eventually separate. Instead of remaining juxtaposed parallelly, as after

colchicine treatment, the chromatids now move away from each other with the centromeres in front of them (Fig. 1 *l*—*q*). The two-armed chromosomes are in this manner bent in the centromeric region just as at the normal anaphase (Fig. 1 *p*—*q*). Since, however, the exterior spindle is lacking, the half-chromosomes will move out irregularly in the plasm in all directions. Finally, it is impossible to identify which half-chromosomes originate from the same chromosome. The two half-chromosomes of the only satellited chromosome present may be recognized, however, and it may then be seen that they are very often lying in diametrically opposite corners of the cell, into which position they must have been forced by the repulsion of their centromeres.

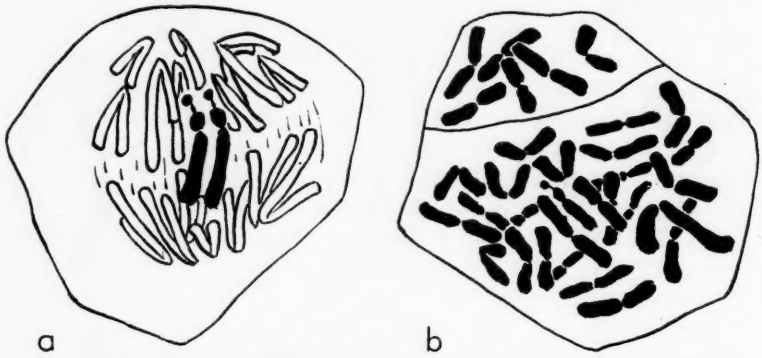


Fig. 2. *Allium fistulosum*, the break-down of the spindle after acenaphthene treatment, *a*: only one chromosome has been affected, *b*: a cell-pair originated from an abnormal anaphase. — × 2500.

As a result of the abnormalities described above a tissue will originate intermingled with cells with abnormal chromosome complements. In the most common case two cells of different size are formed at each telophase, one with more than 2x chromosomes, the other with less. It is often possible at a much later stage to recognize from the cell shape the cell-pairs that have originated in such a manner. These pairs of cells subsequently function as one cell, they enter prophase and pass mitosis together. In Fig. 2 *b* is shown such a pair of cells, which have just started a new mitosis after the preceding affected mitosis. It will be seen that the smaller cell has got 6 chromosomes at the preceding abnormal anaphase, while the larger cell has 26 chromosomes. It is evidently the intimate connection between the two cells which makes it in any way possible for a 6 chromosome cell to complete mitosis. In

pollen grains, for instance, where each cell is more definitely separated from the neighbouring cells, mitosis can never occur in a cell lacking even one single chromosome. Of course, these hypo- and hyperdiploid cells will sooner or later atrophy. And since these cells in the first week of treatment are in absolute majority, this implies a very severe strain on the vitality of the organ in question. This condition is avoided in the more instantaneous action of colchicine.

During the next period of treatment, 4—14 days, c-pairs of the same type as after colchicine treatment are found, and gradually they begin to behave like colchicine induced c-pairs, even on their separation (Fig. 1 *j*—*k*). In other words, the break-down of their centromeres is now complete. And after this the random distribution of the chromosome material of the cells ceases and pure duplications of the chromosomes are brought about. These duplications may be repeated in the same cell provided the effect of the acenaphthene is prolonged. Thus, in root tips treated for 14 days with acenaphthene there occurred numerous polyploid cells, mostly tetraploid but also octoploid and even higher polyploid cells.

The increase of the chromosome number in the cells is accompanied by an increase in the size of the cells, just as after colchicine treatment. This gives rise to the very characteristic swellings of the root tips, the so-called c-tumours. In fact, the c-tumours may be used as a rather sensitive reaction on c-mitoses. It must be remembered, however, that such tumours may be formed also by processes quite different from the c-mitoses, for instance, by treatment with growth substances (LEVAN, 1939). Due to the less complete action of acenaphthene, the c-tumours often become somewhat more extended in length than after colchicine treatment.

When the roots, after the conclusion of the treatment, are transferred into pure water, the spindle apparatus starts functioning again after a time interval of about two days, and after 3—5 days in pure water all mitoses have changed to normal. It is then seen that, in the same way as after colchicine treatment, the mitoses often have a higher chromosome number, 32 or 64, higher up in the root. In the young meristem, on the other hand, very soon normal 16 chromosome mitoses will predominate. This expresses itself macroscopically by the root tip, which grows out after the end of the treatment, rapidly narrowing down to normal thickness. But still after a considerable period in pure water the treated region may be distinguished from the younger part of the root by a more or less abrupt increase in thickness.

II. EXPERIMENTS WITH COLCHICUM.

1. *Material.* — The *Colchicum* species used, *autumnale* L., *Bornmülleri* FREYN., *byzantinum* TEN. and *speciosum* STEV., were procured from the Botanic Gardens of Lund and Copenhagen. Their normal chromosome conditions will be described elsewhere, for the present only a few data being mentioned. The somatic chromosome numbers of the

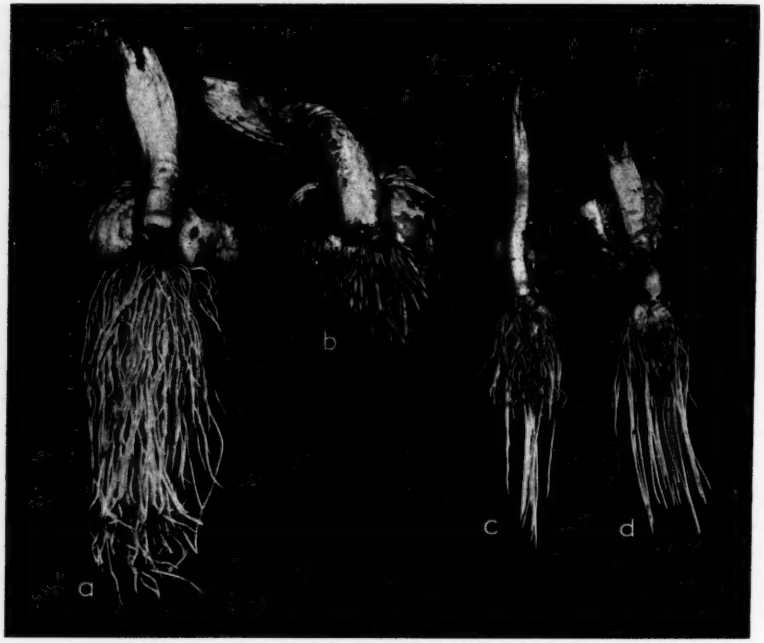


Fig. 3. *a—b*: *Colchicum autumnale*, *c—d*: *Colchicum Bornmülleri*, *b*: roots treated with acenaphthene for 20 days, *d*: roots treated with colchicine solution for 45 days. *a* and *c*: controls in pure water. — Photo H. OLSSON, Svalöf.

Colchicum forms studied so far are situated between 38 and 54. Within the species very considerable differences in chromosome size occur, the longest chromosomes of each idiogram being several times longer than the shortest ones. The absolute length of the chromosomes varies between 0,8 and 6 μ. Most of the chromosomes have submedially located centromeres, but some have their centromeres subterminally—terminally located.

The treatments were made to a great extent in an unheated green-

house during October 1939, and the temperature of the greenhouse was often rather low (about + 2° C.). Root tips were fixed in NAVASHIN, which usually gave very good fixations. A great number of mitoses were present in the roots (sometimes as many as 500 mitoses in one root) and the chromosomes showed very clear centromeric constrictions.

2. *Colchicine treatments.* — Bulbs of the four *Colchicum* species used were placed in colchicine solutions of the concentrations 0,01, 0,1 and 1 %. From 6 hours to 15 days after the beginning of the treatment root tips were fixed at different intervals. And after this time some of the bulbs were left for one month in the solutions. During all this

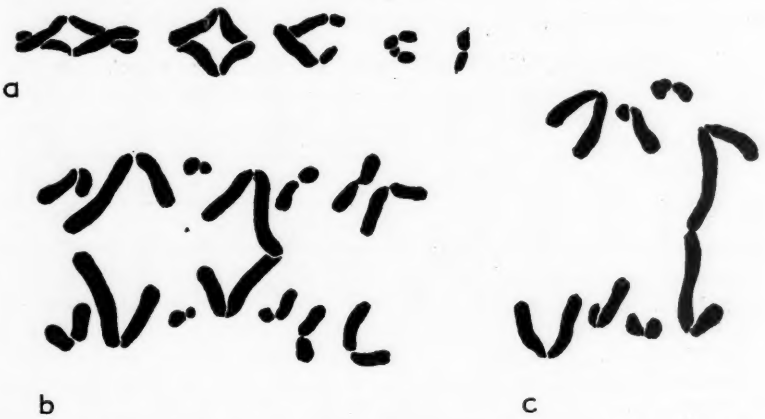


Fig. 4. *Colchicum autumnale*, anaphase chromosomes from a root tip treated for 15 days with 1 % colchicine solution. — × 5000.

time no signs of c-tumours were observed. In *Allium* the formation of c-tumours is quite evident already after a treatment of one day. Fig. 3 *d* shows a *Colchicum* bulb, which was allowed to grow for 1 ½ month in a 1 % colchicine solution, while the bulb pictured in Fig. 3 *c* was left for the same time in pure water. As seen, the colchicine-treated plant has at least as long and vigorous roots as the control plant. As a matter of fact the roots continued to grow rapidly in length all through the period in colchicine.

The cytological study showed no differences whatever in the number and course of the mitoses of the treated plants, as compared with the controls. Even after 15 days in 1 % colchicine no signs of c-mitoses could be detected, and the anaphases took place absolutely normally

(Fig. 4 *a*—*c*). Thus it may be said that the spindle of *Colchicum* is entirely immune to colchicine.

3. *Acenaphthene treatments.* — The 4 species employed all responded very strongly to acenaphthene. The appearance of the macroscopic c-tumour reaction is seen from Fig. 3 *b*, which pictures a bulb, the roots of which had grown for 20 days dusted with acenaphthene crystals. The difference from the control plant in Fig. 3 *a* is striking.

The cytological study of the effect of acenaphthene on the *Colchicum* chromosomes was rendered difficult by the high number of chromosomes present and their small size. It is demonstrated beyond doubt, however, that acenaphthene causes real c-mitoses in *Colchicum* as well as in *Allium*.

TABLE 1. *The course of the acenaphthene effect.*

Species	Period of treatment													
	½ day		1		2		3		4		6		8	
	n[1]	c[1]	n	c	n	c	n	c	n	c	n	c	n	c
Colchicum autumnale		+		+		+	(+)	+		+	(+)	+		+
Colchicum Bornmülleri	+	(+)			+	(+)	+	(+)	+	(+)	+	+	(+)	+
Colchicum byzantinum				+		+		+		+		+		
Colchicum speciosum		+		+		+				+				+

In Table 1 is given a survey of the acenaphthene action on the *Colchicum* mitoses. *Colchicum* seemed to be somewhat more sensitive to acenaphthene than *Allium*. In some cases a number of c-mitoses are found in the treated tissues already after 12 hours. It must be pointed out, however, that the early c-mitoses sometimes looked somewhat abnormal. Thus the c-pairs were often collected into a more or less compact ball in the centre of the cell, and not until after 2—3 days did they begin to assume a more normal appearance. In such cases the acenaphthene treatment seemed to bring about a shock effect, which required some time to fade away. In other cases even after a treatment of 8 days there were a great number of normal mitoses present. mixed with the c-mitoses.

[1] n = normal mitoses, c = c-mitoses.

Another feature also indicating a more sudden response to acenaphthene in *Colchicum* than in *Allium* is the very scarce occurrence of multipolar anaphases. This gave the impression that acenaphthene acts more definitively on the spindle mechanism of *Colchicum* than on that of *Allium*.

Fig. 5 shows the different stages in the development of the c-pairs of *Colchicum*. The chromosomes are scattered disorderly after the disappearance of the nuclear membrane, and the chromatid spiral begins uncoiling (Fig. 5 *a—p*). The variation in size of the *Colchicum* chromosomes, together with differences in the position of their centromeres, gives a very much greater diversity in appearance to the c-pairs than is the case in *Allium*. The centromeres were seldom divided during the

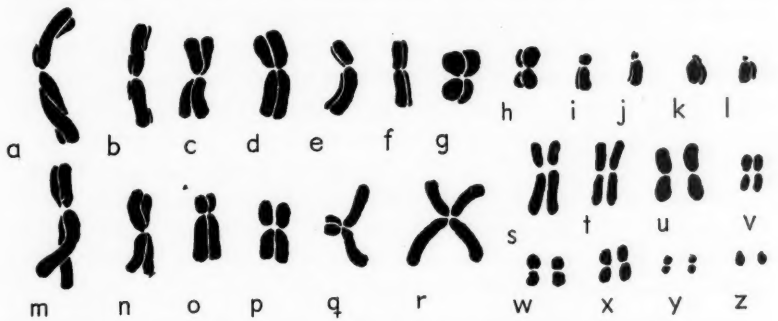


Fig. 5. *Colchicum*, the development of the c-pairs after acenaphthene treatment. — × 4000.

first few days of the treatments, and this accounts for the rare occurrence of multipolar anaphases. When the centromeres on the fourth day after the beginning of the treatment started to divide the centromeres were evidently completely inactivated, so that the half-centromeres did not repel each other (Fig. 5 *s—z*). On the sixth day the second and third c-mitosis in the same cell began to appear. The chromosome numbers could then, without any difficulty be estimated at about 80 and 160 respectively.

The period of spindle recovery could in some cases be followed in detail. After a treatment of four days and a period of one or two days in water single normal mitoses were found among numerous c-mitoses. After a further 5 or 10 days in pure water the same plant showed normal diploid mitoses in the meristem and solitary normal tetraploid mitoses more distant from the tip.

In another case the treatment was continued for 8 days, the recovery being thus controlled after 3, 5 and 7 days in water. In these roots a very high percentage of tetraploid cells and also some octoploid cells were met with. In the cortical cells at a rather long distance from the tip numerous abnormalities occurred even after 7 days in water. One of the most common abnormalities was the occurrence of multinucleate cells, in which the different nuclei entered mitosis simultaneously. These mitoses in the same cell took place without disturbing each other. They often resembled mitoses of the tapetum

TABLE 2. *The occurrence of polyploid mitoses in two roots of Colchicum.*

Distance from the tip	Slide 18142 (acen. 8 days + water 3 days)				Slide 18144 (acen. 8 days + water 5 days)	
	2x	4x	8x	2 or more mitoses in one cell	2x	4x
0—99 μ.....	93	1	—	—	143	7
100—199	120	7	—	—	157	23
200—299	104	2	—	1	72	13
300—399	95	5	—	4	43	29
400—499	66	5	1	6	—	—
500—599	20	2	1	8	—	—
Total	498	22	2	19	415	72

cells. And if two equally-sized nuclei were dividing in one cell the similarity to the second meiotic division was striking. The spindles of the different mitoses might then be orientated either side by side or behind each other in one row.

In some roots the different types of mitoses could be counted section by section. Table 2 gives two instances of such counts. As has previously been demonstrated in *Allium*, it was found also in *Colchicum* that the frequency of polyploid cells increases the greater the distance is from the tip meristem. Thus, as might be expected, the diploid cells of *Colchicum* are superior in the competition with polyploid cells within the mixoploid tissue. Nevertheless, in certain roots whole sectors were found to remain tetraploid during some time after the end of the treatment.

III. DISCUSSION.

My experiments confirm to a great extent the opinion of KOSTOFF that in its effects on mitosis acenaphthene is comparable to colchicine. The negative results with acenaphthene, which have sometimes been reported, must be due either to a special immunity of the experimental plants employed or, more likely, to an erroneous technique. The latter explanation has been suggested by KOSTOFF (1938 b). Differences in the effect of acenaphthene occur, however, in identical material as compared with the effect of colchicine. Thus, acenaphthene acts decidedly more slowly in *Allium* than colchicine. It takes perhaps 1000 times longer to obtain the maximal effect with acenaphthene than with colchicine. The reason of this may be found in the very low degree of solubility of acenaphthene in water, which necessarily retards its absorption in the cell sap. If this is the case, perhaps the external parts of the tissue should be affected first, and this could not be demonstrated in my experiments. The affected cells were found intermingled among normal cells all through the tissue.

On the other hand, the similarity of action between acenaphthene and colchicine is striking. And the final stage of the effect, the completely formed c-pairs, is identical in both cases. The acenaphthene effect, as it appears in *Allium*, may consequently be regarded as an ultra-rapid exposure of colchicine action. And this condition involves, as has been pointed out earlier, a great advantage for the detailed study of the course of the chromosome disturbances. The course of the c-mitosis becomes extremely fractionated.

Great as the advantage of this condition is theoretically, it is equally disadvantageous from a practical viewpoint, when it comes to inducing polyploidy. KOSTOFF emphasizes the lack of poisonous effect of acenaphthene as an advantage, compared with colchicine. This advantage is unfortunately diminished by the slowness in action of the acenaphthene, which must cause a predisposition to the origin of aneuploid cells. Such cells are of course under all conditions formed during the recovery stage. Colchicine, too, gives a slow and fractionated recovery process, during which all kinds of spindle disturbances are found.

It may be mentioned, however, that these conditions have been studied by me in detail only in *Allium*. It is therefore probable that other plants may exhibit another reaction, and will perhaps be to the advantage of acenaphthene in inducing polyploidy. This is of course the case under such special conditions as in *Colchicum*, which is immune

to colchicine. According to BLAKESLEE (l. c.), the fungi are immune to colchicine. In other cases perhaps organisms are found to be hypersensitive to colchicine, so that even small doses cause lethality. Here, too, the less poisonous acenaphthene will be appropriate. It thus follows that from a practical viewpoint it must be important that comparative treatments with different accessible substances are carried out in order to make sure in each case which substance is the most suitable one for the kind of organism under examination.

A very important branch of the intense study of the course of the c-mitosis and the c-meiosis, which has been going on since the discovery of the polyploidy-inducing ability of colchicine, is the analysis of different problems connected with the mechanics of the cell divisions. The possibility of temporarily inactivating the spindle apparatus, affords a valuable opportunity of an experimental approach to the forces governing the normal course of mitosis and meiosis. And I think that the use of acenaphthene may be of still greater importance than colchicine in this field. Thus the differential reaction of the exterior and interior spindle could be very easily studied during the acenaphthene treatment. These two parts of the spindle, conveniently referred to as the centrosomic and centromeric parts, are usually inactivated simultaneously by colchicine. It was only when concentrations just above the threshold value were used (in *Allium* about 0,005 %) that the exterior spindle was regularly inactivated before the interior spindle. Acenaphthene consequently acts as a very dilute colchicine solution, which is in agreement with its very slight solubility in water.

Thus the complex nature of the spindle mechanism is more clearly exhibited by acenaphthene than by colchicine. Beside the difference between the centrosomic and centromeric part of the spindle the centromeric function is further divided into two partial functions: one reproductive function, which brings about the division of the centromere, and one repelling function usually regarded as the internal spindle. The reproductive function is affected by acenaphthene in such a manner that a remarkable delay in the division of the centromeres is brought about, but this function is never arrested completely. The repelling function, on the other hand, is gradually eliminated during the progress of the acenaphthene effect, so that the two daughter chromosomes of each c-pair will eventually remain parallel beside each other.

It is impossible at present to decide if the effect of acenaphthene is really identical with the effect of colchicine. The experiments with *Colchicum* show that the same spindle apparatus can be immune to one

of these substances and susceptible to the other. The possibility touched upon in the introduction, that colchicine and acenaphthene may act upon two different part-functions, both necessary for a normal spindle function, certainly diminishes in probability at the same time as the course of the c-mitoses caused by the two different substances are in detail similar morphologically to each other. A second alternative is that the effect of colchicine and acenaphthene are identical, the observed differences in their action being due just to differences in solubility, in reactivity etc. This second alternative necessitates the assumption of the presence of an antivenom in *Colchicum* (cf. BLAKESLEE, l. c.), which inactivates the colchicine in the cells, before the colchicine action is brought about. The genus *Colchicum* must necessarily have aquired the faculty of destroying colchicine before the faculty of producing colchicine.

SUMMARY.

The effect of acenaphthene and colchicine is studied on root mitoses of *Allium* and *Colchicum*.

In *Allium* acenaphthene is found to bring about the same deviation from normal mitosis as colchicine. The slower and less complete action of acenaphthene, as compared with colchicine, may be ascribed to its lower degree of solubility in water.

The differential reaction of one exterior and one interior part of the spindle apparatus already observed after colchicine treatment is made more evident after acenaphthene treatment.

Colchicum is shown to be entirely immune to colchicine, but highly susceptible to acenaphthene, which causes regular c-mitoses in *Colchicum*, giving rise to tetraploid and octoploid cells and sectors.

Svalöf, 14th November 1939.

LITERATURE CITED.

1. BLAKESLEE, A. F. 1939. The present and potential service of chemistry to plant breeding. — Am. Journ. of Bot. **26**: 163—172.
2. KOSTOFF, D. 1938 a. Irregularities in the mitosis and polyploidy induced by colchicine and acenaphthene. — C. R. (Doklady) Acad. Sci. U.R.S.S. **19**: 197—199.
3. — 1938 b. Colchicine and acenaphthene as polyploidizing agents. — Nature **142**: 753.

4. LEVAN, A. 1938. The effect of colchicine on root mitoses in *Allium*. — Hereditas XXIV: 471—486.
5. — 1939. Cytological phenomena connected with the root swelling caused by growth substances. — Hereditas XXV: 87—96.
6. NAVASHIN, M. 1938. Influence of acenaphthene on the division of cells and nuclei. — C. R. (Doklady) Acad. Sci. U.R.S.S. 19: 193—196.
7. NEBEL, B. R. 1938. Colchicine and acenaphthene as polyploidizing agents. — Nature 142: 257.
8. SCHMUCK, A. 1938. The chemical nature of substances inducing polyploidy in plants. — C. R. (Doklady) Acad. Sci. U.R.S.S. 19: 189—192.
9. SCHMUCK, A. and KOSTOFF, D. 1939. Brome-acenaphthene and brome-naphthaline as agents inducing chromosome doubling in rye and wheat. — C. R. (Doklady) Acad. Sci. U.R.S.S. 23: 263—266.
10. SIMONET, M. et GUINOCHET, M. 1939. Anomalies morphologiques et caryologiques provoquées, sur les jeunes plantules, par les dérivés halogenes des carbures cycliques. — C. R. Soc. Biol. 131,: 222—224.

ZUR GENETIK VON PHASEOLUS VULGARIS

XVI. WEITERE BEITRÄGE ZUR VERERBUNG DER TEILFARBIGKEIT

VON *HERBERT LAMPRECHT*
SAATZUCHTANSTALT WEIBULLSHOLM, LANDSKRONA

(With a Summary in English)

VOR einigen Jahren habe ich (LAMPRECHT, 1934) eine Arbeit über die Vererbung der Teilfarbigkeit der Testa bei *Phaseolus vulgaris* veröffentlicht, in der teils die bis dahin auf diesem Gebiet bekannten Resultate zusammenfassend besprochen, teils die Spaltungsergebnisse in F_2 von sieben Kreuzungen mitgeteilt wurden. Das bis dahin Bekannte und die Ergebnisse dieser Arbeit können etwa folgendermassen kurz zusammengefasst werden.

Für die Ausbildung von Teilfarbigkeit ist vor allem Rezessivität in den zwei Grundgenen, *T* und *E*, erforderlich. Alle *TE*-Pflanzen sind ganzfarbig. Ausserdem gibt es mehrere Teilfarbigkeitsgene, die je für sich, wie auch zusammen, die Färbung verschiedener Gebiete der Testa bedingen dürften. Zwischen diesen Genen besteht Komplexwirkung. Welches Aussehen Samen haben, die in allen Teilfarbigkeitsgenen rezessiv sind, ist bisher unbekannt. Es wäre demnach denkbar, dass die Grundgene allein schon einen gewissen Typus von Teilfarbigkeit bedingen könnten.

In der genannten Arbeit wurden 17 erblich verschiedene Typen von Teilfarbigkeit beschrieben und abgebildet. Die l. c. eingeführte Bezeichnung der Typen erfolgt mit lateinischen Namen, die die Gestalt der Zeichnung auf der Samenschale angeben. So z. B. *bipunctata* = mit zwei kleinen Flecken, *virgata* = mit Streifen, *arcus* = mit Bogen, *virg-arcus* = mit Streifen und Bogen, *sellatus* = Sattel u. s. w. (vgl. die weiter unten folgenden Abbildungen). Im ganzen waren schon damals etwa 22 verschiedene Typen von Teilfarbigkeit bekannt, weshalb angenommen wurde, dass vier, wahrscheinlicher fünf, verschiedene Gene für Teilfarbigkeit bestehen.

Die Aufspaltung in den mitgeteilten Kreuzungen war, ausgenommen in einer Kreuzung, Nr. 32, unklar. Die erhaltenen Spaltungszahlen für die verschiedenen teilfarbigen Typen liessen sich nicht durch die bekannten einfacheren Schemas für 2- oder 3-Genenspaltung erklären.

Zwei Ursachen dürften hierzu wesentlich beigetragen haben. Teils waren alle Kreuzungen zwischen ganzfarbigen und teilfarbigen Linien ausgeführt, wobei sich herausstellte, dass die meisten ganzfarbigen in der genotypischen Konstitution für Teilfarbigkeit Unterschiede in mehreren Genen aufwiesen. Teils war die Klassifikation der teilfarbigen Typen in gewisser Hinsicht unsicher. Es gab allerdings kaum ganz kontinuierliche Übergänge zwischen den weniger gefärbten Typen — zwischen *unipunctata* und *virgarcus* liegend — aber bei nicht wenigen von diesen war ein gewisser Teil der Teilfarbigkeit häufig schwach ausgebildet. Dies wurde damals als durch modifikative Einflüsse verursacht aufgefasst. Wie unten gezeigt wird, beruhen diese Erscheinungen in der Hauptsache auf Heterozygotie in den Genen für Teilfarbigkeit.

Genpaare für Teilfarbigkeit sind bisher erst zwei in ihrem Effekt näher charakterisiert. Das eine Genpaar ist *Z—z* und bezieht sich auf die drei Typen sellatus, *ZZ*, Piebald, *Zz*, und virgarcus, *zz*. Belege für diese Spaltung stammen von E. v. TSCHERMAK (1912), SURFACE (1916) sowie SAX und MCPHEE (1923). Das zweite Genpaar wurde vom Verfasser (LAMPRECHT, 1934) aufgestellt und abgeleitet von *bipunctata* mit *Bip—bip* bezeichnet. Es ist eines der Gene, die für die Spaltung *virgarcus* (dominant) : *bipunctata* (rezessiv) (mit Zwischentypen) verantwortlich sind (vgl. unten Fig. 1 und 2).

Seither hat F. SCHREIBER (1934) eine Arbeit veröffentlicht, laut der es einen dominanten »Löschfaktor» für Teilfarbigkeit gibt. Dieser sollte also die Ausbildung von Teilfarbigkeit ganz verhindern, sodass anstatt teilfarbigen, reinweisse Samen resultieren. Selbst habe ich auch in mehreren Kreuzungen (siehe l. c.) die Ausspaltung von weisssamigen aus teilfarbigen Pflanzen beobachtet, ohne jedoch hierbei ein über Teilfarbigkeit allgemein dominierendes Gen gefunden zu haben.

KREUZUNG BIPUNCTATA- × VIRGATA-TYPUS.

Diese Kreuzung, Nr. 253, wurde ausgeführt zwischen Linie 5 vom *bipunctata*-Typus, herstammend aus der französischen Brechbohnensorte *Incomparable*, und Linie 88 vom *virgata*-Typus, ausgelesen aus der englischen Brechbohnensorte *Early Giant*. Der *bipunctata*-Typus ist in Fig. 1 links, der *virgata*-Typus in Fig. 1 rechts abgebildet. Beide Typen zeigen eine gewisse, aber doch nur relativ geringe Variation (vgl. Fig. 9 in LAMPRECHT, 1934, wo 3 Samen abgebildet sind, die etwa die Variationsbreite von L. 5 angeben, sowie Fig. 19 l. c., in der der mittlere und rechte Samen dem *virgata*-Typus von L. 88 entsprechen).

Die auf den F_1-Pflanzen erhaltenen Samen zeigten die Zeichnung des mittleren Samens in Fig. 1. Wie ersichtlich hat dieser die beiden für den *bipunctata*-Typus charakteristischen zwei Flecken gleich entwickelt wie bei der Elternlinie, d. h. sie zeigten die gleiche Variationsbreite wie bei dieser. Anders verhält es sich mit dem auf der Mikropylenseite befindlichen Streifen des *virgata*-Samens. Dieser ist hier nicht voll ausgebildet sondern nur durch Punkte angegeben, die allerdings mitunter mehr oder weniger miteinander verfliessen können. Man konnte schon unter den auf F_1 erhaltenen Samen solche antreffen, in denen es schwer erschien sie sicher von schwach ausgebildeten *virgata* der Elternlinie zu unterscheiden.

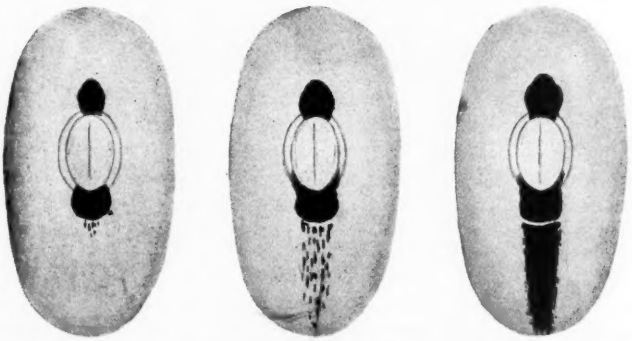


Fig. 1. Links ein Same vom *bipunctata*-Typus, *bip arc*, rechts ein solcher vom *virgata*-Typus, *bip Arc*. Der mittlere Same entspricht der heterozygoten Konstitution *bipbip Arcarc* (F_1 von Kreuzung Nr. 253).

In der zweiten Generation wurden 600 Samen gesät, die im ganzen 537 voll reifende Pflanzen entwickelten. Die Klassifikation der *bipunctata*-Samen verursachte keinerlei Schwierigkeit, sie waren leicht von den übrigen zu scheiden. Bei den *virgata*-Samen dagegen erschien es schwierig eine bestimmte Grenze zu ziehen zwischen voll ausgebildeten *virgata*-Typen (= Elternlinie 88) und solchen mit nur durch Punkten angedeutetem Streifen (vgl. LAMPRECHT, 1934, Fig. 19, linker und mittlerer Samen). Die zwischen den letztgenannten beiden Typen gezogene Grenze ist also nicht scharf. Für die drei Typen in F_2 ergaben sich folgende Zahlen:

Gefunden:	132	*bipunctata*:	294	*schwach virgata*:	111	*typisch virgata*		
Erwartet:	134,25	»	: 268,50	»	»	: 134,25	»	»
D/m für 1 : 2 : 1 =	0,22		2,20			2,31		

Es besteht kein Zweifel, dass es sich hier um eine monohybride Spaltung nach dem *Zea*-Typus handelt, bei der die Abgrenzung der heterozygot von den homozygot *virgata* nicht ganz sicher erscheint. In diesem Gebiet gibt es Zwischentypen, deren Zugehörigkeit erst durch Untersuchung in einer weiteren Generation sichergestellt werden kann. Die F_3-Generation hat dies auch bestätigt. Es wurden insgesamt 22 Familien untersucht. Sämtliche *bipunctata*-Typen, 6 Familien mit zusammen 109 Individuen, haben nur wiederum solche Nachkommen gegeben. Und das Gleiche war mit den Nachkommen nach typischen *virgata* der Fall, 5 Familien mit 92 Pflanzen verblieben konstant *virgata*. Von den 11 Familien, die nach *schwach virgata* gebaut wurden, waren dagegen 2 konstant *virgata*, jetzt mit deutlich ausgebildetem *virgata*-Streifen,

Fig. 2. Drei Samen vom *virgarcus*-Typus der Linie 57 aus der Sorte Goldregen. Formel: *Bip Arc.* Der linke Same hat ungewöhnlich schwach ausgebildeten *arcus*.

während die übrigen 9 Familien im Verhältnis 51 *bipunctata* : 92 *schwach virgata* : 34 *typisch virgata* spalteten. Hier liegt also offenbar wiederum dasselbe 1 : 2 : 1-Verhältnis wie in F_2 vor.

Für den Unterschied zwischen *virgata* und *bipunctata* ist demnach ein Gen verantwortlich zu machen, das in seiner dominanten Form die *virgata*-, in seiner rezessiven die *bipunctata*-Zeichnung bedingt. Ein Symbol für dieses Gen wird bei Besprechung der nächsten Kreuzung eingeführt werden.

KREUZUNG BIPUNCTATA- × VIRGARCUS-TYPUS.

Diese Kreuzung, Nr. 251, wurde ausgeführt zwischen Linie 5 (wie in voriger Kreuzung) und Linie 57, aus der deutschen Wachsbohnensorte Goldregen, die den *virgarcus*-Typus repräsentiert. Die Samen des letzteren zeigen die in Fig. 2 wiedergegebene Zeichnung und Variation.

Die auf F_1 erhaltenen Samen zeigten die Zeichnung des rechten

Samens in Fig. 4. Die Ausbildung des *virgata*-Streifens sowie des vom oberen Fleck nach beiden Seiten des Hilums ausgehenden Bogens variierte in der Stärke, erreichte aber niemals diejenige der Elternlinie

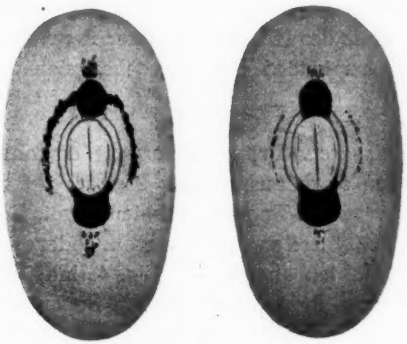


Fig. 3. Links *arcus*-Typus homozygot und gut ausgebildet: *BipBip arcarc*; rechts *arcus*-Typus heterozygot: *Bipbip arcarc*.

57 (Fig. 2). Mitunter waren diese beiden Abzeichen schwach ausgebildet, aber doch stets noch erkennbar.

Die zweite Generation bestand aus 552 Individuen (gesät 600

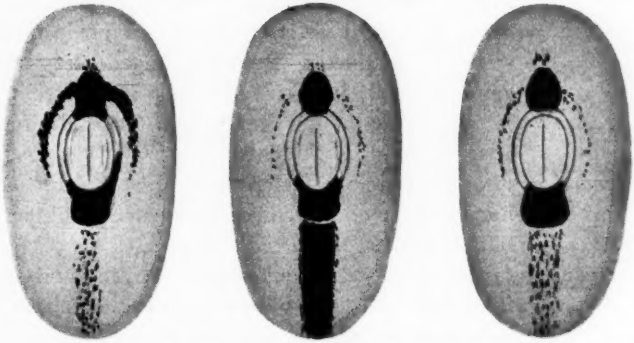


Fig. 4. Drei verschieden heterozygote *virgarcus*-Typen; links *virgata*-heterozygot: *BipBip Arcarc*; Mitte *arcus*-heterozygot: *Bipbip ArcArc*; rechts *virgata*- und *arcus*-heterozygot: *Bipbip Arcarc*.

Samen). Die auf dieser erhaltenen Samen wurden in neun verschiedene Gruppen eingeteilt, entsprechend den neun verschiedenen, möglichen Genkombinationen einer bifaktoriellen Spaltung. Die Farbenverteilungen auf der Testa und die diesen entsprechenden Genkonstitutionen ergeben sich aus den Fig. 1—4.

Das eine der beiden hier wirksamen Gene ist das bereits früher bekannte Gen *Bip* (LAMPRECHT, 1934). Das zweite bezeichne ich mit *Arc*, abgeleitet von *arcus* = Bogen, Fig. 3 links. Diese, der sog. *arcus*-Typus, der in *arc* doppeltrezessiv ist, wird also durch einen farbigen Bogen zu beiden Seiten des Hilums charakterisiert. Zwischen Teilfarbigkeitstypen und diesen beiden Genen ergeben sich dann folgende Beziehungen.

BipBip ArcArc = *virgarcus*-Typus homozygot, sowohl Bogen wie Streifen stark ausgebildet; Fig. 2.

BipBip Arcarc = *virgarcus*-Typus *Arc* heterozygot, Bogen gut ausgebildet, Streifen nur in mehr oder weniger deutlichen Punkten angedeutet; Fig. 4 links.

BipBip arcarc = *arcus*-Typus homozygot, Bogen schön ausgebildet, Streifen fehlt; Fig. 3 links.

Bipbip ArcArc = *virgarcus*-Typus *Bip*-heterozygot, Streifen stark ausgebildet, Bogen nur in Punkten angedeutet; Fig. 4 Mitte.

Bipbip Arcarc = *virgarcus*-Typus *Arc*- und *Bip*-heterozygot, sowohl Bogen wie Streifen nur in mehr oder weniger deutlichen Punkten angedeutet; Fig. 4 rechts.

Bipbip arcarc = *arcus*-Typus *Bip*-heterozygot, Bogen nur in mehr oder weniger deutlichen Punkten angedeutet (mitunter ganz fehlend), Streifen fehlt stets; Fig. 3 rechts.

bipbip ArcArc = *virgata*-Typus homozygot, Bogen fehlt, Streifen stark ausgebildet; Fig. 1 rechts.

bipbip Arcarc = *virgata*-Typus *Arc*-heterozygot, Bogen fehlt, Streifen nur in mehr oder weniger deutlichen Punkten ausgebildet; Fig. 1 Mitte.

bipbip arcarc = *bipunctata*-Typus homozygot, Bogen und Streifen fehlt, nur die beiden Flecken an Caruncula und Mikropyle sind vorhanden; Fig. 1 links.

Aus den Figuren ist ferner ersichtlich, dass auch beim *bipunctata*- und beim *arcus*-Typus einige Farbpunkte des Streifens vorkommen können, aber bei diesen Typen befinden sich diese dann nur in unmittelbarer Nähe der beiden Flecken an Caruncula und Mikropyle (vgl. Fig. 1 links und 3). Eine Verwechslung mit dem *virgata*-Typus erscheint hierdurch kaum möglich (vgl. Fig. 1 Mitte und 4 links und rechts).

Die Verteilung der 552 Pflanzen der F_2-Generation auf die oben angeführten neun Gruppen war die folgende:

		Gefunden:	Theoretisch erwartet:
virgarcus-	*BipBip ArcArc* konstant *virgarcus*	35	34,5
Gruppe	*BipBip Arcarc virgata*-spaltend	16	69,0
	Bipbip ArcArc arcus-spaltend	78	69,0
	Bipbip Arcarc virgata-u. *arcus*-spaltend	205	138,0
	S:a	334	310,5
arcus-	*BipBip arcarc* konstant *arcus*	2	34,5
Gruppe	*Bipbip arcarc arcus*-spaltend	33	69,0
	S:a	35	103,5
virgata-	*bipbip ArcArc* konstant *virgata*	14	34,5
Gruppe	*bipbip Arcarc virgata*-spaltend	93	69,0
	S:a	107	103,5
bipunctata-			
Gruppe	*bipbip arcarc* konstant *bipunctata* S:a	76	34,5

Bei Betrachtung dieser Zahlen kann folgendes festgestellt werden. Die gefundenen Individuensummen für die beiden Gruppen *virgarcus* und *virgata* zeigen mit den bei Zweigenenspaltung erwarteten gute Übereinstimmung. In der *arcus*-Gruppe besteht dagegen ein starkes Defizit und in der *bipunctata*-Gruppe ein etwa dementsprechender Überschuss. Vereinigt man diese beiden Gruppen, so resultieren folgende Zahlen.

Gefunden:	334	*virgarcus* :	107	*virgata* :	111	*arcus-bipunctata*
Erwartet:	310,5	» :	103,5	» :	138,0	» »
D/m für						
9 : 3 : 4 =	2,02		0,49		2,66	

Es handelt sich also zweifellos um eine bifaktorielle Spaltung in den beiden Genpaaren *Bip—bip* und *Arc—arc*, nur lässt die Abgrenzung der verschiedenen heterozygoten Gruppen zu wünschen übrig. Innerhalb jeder der beiden Gruppen *virgarcus* und *virgata* finden wir wiederum dieselbe Unsicherheit bei der Abgrenzung des homozygoten gegen den heterozygoten Streifen wie in der vorigen Kreuzung, nur hier in noch stärkerem Masse. Ferner ist die Ausbildung des *arcus* meistens schlecht.

Nur 2 Individuen wurden als homozygot *arcus* und 33 als heterozygot klassifiziert. Der Rest, etwa 60 Individuen, kam in die *bipunctata*-Gruppe. Dies bewies die dritte Generation, in der aus *bipunctata* sowohl hetero- wie homozygote *arcus*-Typen ausspalteten. Da die Ausbildung des *arcus* sowohl in Linien wie in anderen Kreuzungen jedoch eine gute sein konnte, erscheint es möglich, dass in Kr. 251 noch ein Gen wirkt, das die schwache und unsichere Ausbildung dieses Typus bedingt. Wahrscheinlich handelt es sich hierbei um ein Teilfarbigkeitsgen, das beide Elternlinien in gleicher Allelenform enthalten.

KREUZUNG BIPUNCTATA- × MAJOR-TYPUS.

Diese Kreuzung, Nr. 174, wurde ausgeführt zwischen L. 5 (wie in voriger Kreuzung) und L. 6 aus der französischen Brechbohnensorte

TABELLE 1. *Die Aufspaltung des Bastards Arcarc Bipbip Diffdiff in F_2 von Kreuzung Nr. 174.*

Genenspaltung				Teilfarbigkeitstypus	Individuenanzahl		D/m
					Gefunden	Erwartet	
F_1 :	48 *Arc*	36 *Bip*	27 *Diff*	*virgarcus*	238	231,19	0,59
			9 *diff*	*maximus*	66	77,06	1,36
		12 *bip*	9 *Diff*	*virgata*	60	77,06	2,10
			3 *diff*	*major*	29	25,69	0,67
	16 *arc*	12 *Bip*	(*Diff*/*diff*)	*arcus*	155	137,00	1,77
		4 *bip*	(*Diff*/*diff*)	*bipunctata*			

Très nain précoce. Letztere repräsentiert den *major*-Typus; siehe Fig. 5. Die auf der ersten Generation erhaltenen Samen zeigten die in Fig. 6 wiedergegebene Zeichnung. Wie ersichtlich stehen diese Samen dem *virgarcus*-Typus nahe, doch mit etwas stärkerer Ausbildung des *arcus* und zweier Lappen an den Seiten der Mikropyle. In den beiden vorigen Kreuzungen zeigte sich, dass Heterozygotie in den beiden Genen für Teilfarbigkeit *Arc* und *Bip* intermediäre Typen ergab. Dasselbe scheint auch hier der Fall zu sein, jedoch mit einer starken Annäherung an den Typus mit geringerer Ausbreitung von Farbe auf der Testa. Die grössere Ausbreitung der Farbe, wie sie der eine Elter (Linie 6) zeigt, scheint

daher durch Rezessivität in einem weiteren Teilfarbigkeitsgen bedingt zu werden.

In der zweiten Generation wurden 600 Samen gesät, die 548 samentragende Pflanzen entwickelten. Diese konnten hinsichtlich Teilfarbig-

Fig. 5. Drei Samen vom *major*-Typus aus Linie 6, Très nain précoce. Der eine Elter von Kreuzung Nr. 174.

keit hauptsächlich in folgende 6 verschiedene Typen eingeteilt werden: *bipunctata, arcus, virgata, virgarcus, maximus* und *major* (vgl. Fig. 1—5 und 7). Die für diese sechs Typen gefundenen und theoretisch erwarteten Individuenanzahlen sind in Tabelle 1 zusammengestellt. Aus dieser

Fig. 6. Die Zeichnung der auf F_1 von Kreuzung Nr. 174 erhaltenen Samen; Formel: *Bipbip Arcarc Diffdiff.*

ist vor allem ersichtlich, dass teils eine Spaltung in den beiden in voriger Kreuzung behandelten Genen *Arc* und *Bip*, teils eine Spaltung in einem weiteren Gen stattgefunden hat, das als *Diff* bezeichnet wurde. Da nun die eine Elternlinie, Nr 5, vom *bipunctata*-Typus in *Arc* und *Bip* rezessiv ist (vgl. die vorige Kreuzung), kann auf Grund der gefundenen

Spaltung geschlossen werden, dass der durch die andere Elternlinie, Nr. 6, repräsentierte *major*-Typus in diesen beiden Genen dominant sein muss. Ferner muss sich der *major*-Typus in wenigstens noch einem weiteren Gen vom *bipunctata*-Typus unterscheiden. Dieses neue Gen bedingt laut den Spaltungsresultaten in rezessiver Form eine Ausbreitung der Farbe auf der Testa. Mit Hinblick hierauf wurde es mit dem Symbol *diff*, abgeleitet von *diffundere* = ausbreiten, belegt.

In den beiden Genpaaren *Arc—arc* und *Bip—bip* erhalten wir die gleiche Spaltung wie in der vorigen Kreuzung, nämlich dem Verhältnis 9 : 3 : 4 entsprechend, da auch hier wiederum die Grenze zwischen den beiden Typen *arcus* und *bipunctata* nicht scharf ist. Der *arcus* ist oft sehr schwach ausgebildet oder nicht mehr erkennbar, sodass ein Teil

Fig. 7. Drei Samen vom *maximus*-Typus.

der *arcus*-Typen als *bipunctata* klassifiziert werden. Für die Spaltung in diesen beiden Genen ergaben sich folgende Zahlen:

Gefunden:	304	*Arc Bip* :	89	*Arc bip* :	155	*arc* $\binom{Bip}{bip}$
Erwartet:	308,25	» » :	102,75	» » :	137,00	» »
D/m für 9 : 3 : 4 =	0,37		1,51		1,77	

Wie ersichtlich ist die Übereinstimmung zwischen gefundenen und theoretisch erwarteten Zahlen befriedigend. Von den *arc*-Individuen wurden 72 als *arcus* und 83 als *bipunctata* klassifiziert, was auf die vorhin erwähnte schlechte Ausbildung des *arcus* zurückzuführen ist.

Das neue Gen *diff* scheint bei Rezessivität in *arc*, wenigstens bei den hier in Frage kommenden genotypischen Konstitution, weder in dominanter noch in rezessiver Form einen Einfluss zu besitzen. In der Gruppe der *Arc*-Individuen bedingt Rezessivität in *diff* eine starke Ausbreitung

der Teilfarbigkeit. Der *virgarcus*-Typus wird durch *diff* in den *maximus-*, der *virgata*-Typus in den *major*-Typus umgewandelt. Es kann hier die nicht häufige Erscheinung festgestellt werden, dass gewisse Gene für eine Eigenschaft (Teilfarbigkeit) diese in dominanter Form verstärken, während ein anderes Gen (*diff*) dies in rezessiver Form bedingt. D. h. *Arc* und *Bip* verursachen in dominanter Form grössere Ausbreitung der Farbe auf der Testa, *Diff* bedingt grössere Ausbreitung dagegen in rezessiver Form.

Wie schon für F_1 erwähnt worden ist, zeigen *virgarcus*-Samen bei Heterozygotie in *Diff* ein wenig grössere Verbreitung der Farbe (s. Fig. 6). Dasselbe scheint auch für den *virgata*-Typus zu gelten. Auch

Fig. 8. In Kreuzung 174 ausgespaltene Samen vom *major-* und *maximus*-Typus, die in einem oder mehreren Genen heterozygot sind. Links *major*-Typus, der in *virgata* (*Bipbip*) und vielleicht auch in *arcus* heterozygot ist, in der Mitte ein Same mit homozygot *virgata*, aber wahrscheinlich heterozygot *arcus*. Rechts *maximus*-Same mit heterozygot *virgata* und wahrscheinlich auch *arcus*.

bei den Samen vom *maximus-* und *major*-Typus, die also beide in *diff* homozygot rezessiv sind, kann Heterozygotie in *Arc* und *Bip* an der Zeichnung festgestellt werden. Als Beispiel hierfür soll Fig. 8 dienen. Wie aus dieser ersichtlich, wird die Ausbreitung der Farbe bei Heterozygotie in *Bip* offenbar vermindert. Heterozygotie in *Arc*, die den Streifen des *virgata*-Typus in Punkte auflöst, kann, wie der linke und rechte Samen in der Fig. zeigen, leicht festgestellt werden. Beim mittleren Samen geht der Streifen breit und stark um das Ende des Samens herum; er ist hier deutlich homozygot, *ArcArc*. Die durch das Gen *diff* zu beiden Seiten der Mikropyle bedingten Lappen scheinen stets deutlich ausgebildet zu werden (vgl. z. B. Fig. 8 rechter Samen).

Eine scharfe Abgrenzung aller dieser verschiedenen heterozygoten Typen gegeneinander ist ziemlich schwierig und wahrscheinlich nur

dann sicher durchzuführen, wenn Spaltung nur in einem der betreffenden Gene stattfindet. Hierzu sind also weitere Analysen in F_3 und F_4 usw. bzw. in speziell hierauf abzielenden Kreuzungen erforderlich.

KREUZUNG VIRGARCUS- × MINOR-TYPUS.

Als *virgarcus*-Typus wurde zu dieser Kreuzung Linie 57, aus der deutschen Wachsbohnensorte Goldregen stammend, benutzt, die auch

Fig. 9. Drei Samen vom *minor*-Typus, Linie 106, der eine Elter von Kreuzung Nr. 282.

zu Kr. 251 als Elter verwendet worden ist. Der zweite Elter, Linie 106, stammt wahrscheinlich von einer spontanen Kreuzung und wurde als

Fig. 10. Drei Samen vom *minimus*-Typus, Linie 63.

Einmischung in einer Samenprobe angetroffen. Bei Vermehrung seiner Nachkommen zeigte sich, dass diese hinsichtlich Teilfarbigkeit konstant waren. Die Testafarbe ist Kastanienbraun, Formel: *P C J G b V r t*. Der *minor*-Typus (vgl. Fig. 9) nimmt eine intermediäre Stellung zwischen dem *major*- und dem *minimus*-Typus ein (vgl. Fig. 10). Ohne sich von der erblich bedingten Konstanz des *minor*-Typus überzeugt zu haben, wäre man am ehesten geneigt, denselben als eine Modifikation des *major*-Typus anzusprechen, bei dem die Farbe eine etwas grössere Ver-

breitung erhalten hat. Er steht nämlich dem *major*-Typus in der Ausbreitung der Farbe etwas näher als dem *minimus*-Typus.

Die auf F_1 dieser Kreuzung erhaltenen Samen zeigten die in Fig. 11 wiedergegebene Zeichnung. In der Ausbreitung der Farbe entsprachen sie also am ehesten dem *major*-Typus, unterscheiden sich aber von diesem bestimmt durch die zu beiden Seiten des Streifens (*virgata*) gegen die Mikropyle gerichteten hellen Einschnitte. Beim *major*-Typus fehlen überdies auch die hier oberhalb der Lappen vorhandenen hellen Einschnitte. Da der Streifen (*virgata*) voll und stark ausgebildet ist, sollen die Samen *ArcArc* sein. Es wäre demnach zu erwarten, dass der eine Elter, L. 106, der *minor*-Typus *ArcArc* in seiner genotypischen Konstitution hat. F_2 hat dies auch bestätigt.

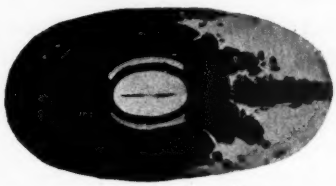


Fig. 11. Auf F_1 von Kreuzung Nr. 282 erhaltener Same. Formel: *Bipbip ArcArc Diffdiff Expexp.*

Die zweite Generation spaltete ausser in den Typen *virgata* und *virgarcus* in einer Serie von Typen, die hinsichtlich Ausbreitung der Farbe alle Übergänge vom *maximus*- bis zum *minimus*-Typus aufweist. Eine Abgrenzung der vier Typen *maximus, major, minor* und *minimus* sowie ihrer Heterozygoten, entsprechend ihrer genotypischen Konstitution ist daher nicht möglich gewesen. Die folgende Übersicht über die erhaltenen Typen ist also nur als Orientierung zu betrachten und können die gefundenen Zahlen nicht zu einer zahlenmässigen Bearbeitung der Spaltungsverhältnisse verwendet werden. Es wurden gefunden:

Teilfarbigkeits-Typus	Anzahl Individuen
virgata	28
virgarcus, heterozygot in *arcus*	96
virgarcus	38
maximus, mit geringerer Farbenausbreitung	79
maximus	45
major, mit geringerer Farbenausbreitung	66
major	52
minor, mit geringerer Farbenausbreitung	41
minor	47
minimus, mit geringerer Farbenausbreitung	56
minimus	26
Summe:	574

Wenn also die vorstehend angeführten Zahlen auch zu einer exakten Analyse der stattgefundenen Spaltung ganz ungeeignet sind, so lassen sie doch in bezug auf die beteiligten Gene einen recht sicheren Schlusssatz zu. In der vorigen Kreuzung, Nr. 174, wurde ein Gen *diff* festgestellt, das in rezessiver Form den *virgata*- in den *major*-Typus und den *virgarcus*- in den *maximus*-Typus umwandelte. Dieselben Typen werden auch in vorliegender Kreuzung gefunden. Es liegt demnach Spaltung in den beiden Genen *Bip* und *Diff* vor. Es wurden aber überdies noch zwei weitere Typen von Teilfarbigkeit, nämlich *minor* und *minimus,* beobachtet. Die erbliche Konstanz dieser Typen ist schon früher festgestellt worden (LAMPRECHT, 1934); d. h. also, dass sie in homozygoter Form auftreten. In vorliegender Kreuzung muss also noch ein Gen spalten, das für die Ausbildung dieser beiden Typen verantwortlich ist und das offenbar durch den einen teilfarbigen Elter, den *minor*-Typus, eingeführt worden ist. Da die beiden teilfarbigen Typen mit grösster Ausbreitung von Farbe auf der Testa, der *minor*- und der *minimus*-Typus, in geringerer Anzahl auftreten als der *maximus*- und der *major*-Typus, sowie da der *minimus*-Typus, der die grösste Ausbreitung der Farbe aufweist, überdies am seltensten ist, ist die Wirkung des hier in Frage stehenden Gens offenbar eine dem Gen *diff* entsprechende, d. h. es bedingt gleichwie dieses in rezessiver Form grössere Ausbreitung der Farbe. Das hierfür verantwortliche Gen sei, abgeleitet von *expandere* = ausbreiten, mit dem Symbol *Exp* belegt.

Eine Abgrenzung der verschiedenen Heterozygoten gegenüber den Homozygoten in der Typenserie *maximus—major—minor—minimus* wird nur in Kreuzungen mit Spaltung in je einem einzigen Gen für Teilfarbigkeit, und auch dann vielleicht nur durch quantitative Methoden, möglich sein.

SUMMARY.

1. To increase our knowledge of the inheritance of partly colouring of the seed coat of *Phaseolus vulgaris* four crosses were studied.

2. The two types of partly colouring *bipunctata* and *virgata* (Fig. 1) differ in the gene *Arc*. The genotypical constitution of the *bipunctata*-type is *bipbip arcarc,* that of the *virgata*-type is *bipbip ArcArc.* Heterozygosity in *Arc* causes the formation of an intermediate type (Fig. 1).

3. Through yet another gene, *Bip*, in co-operation with *Arc*, the following types of partly colouring arise: *arcus, BipBip arcarc* (Fig. 3), *virgarcus, BipBip ArcArc* (Fig. 2). Heterozygosity in the gene *Bip* also causes intermediate types (Fig. 4).

4. While the two genes *Arc* and *Bip* in their *dominant* state cause the extension of colour on partly coloured types, the two new genes *Diff* and *Exp* do so in their *recessive* state. These two genes *Diff* and *Exp* are responsible for the formation of the types: *maximus, major, minor* and *minimus* (Figs. 5, 7, 9, 10). Heterozygosity in these genes causes also intermediate types (Figs. 6, 8, 11). In such cases the result will be a series of gradual transitions, as e. g. in cross No. 282 from *maximus* to *minimus*.

ZITIERTE LITERATUR.

1. LAMPRECHT, H. 1934. Zur Genetik von *Phaseolus vulgaris*, VIII. Über Farbenverteilung und Vererbung der Teilfarbigkeit der Testa. — Hereditas, XIX, 177—222.
2. SAX, K. and MCPHEE, H. C. 1923. Color Factors in Bean Hybrids. — Journ. of Heredity, XIV, 205—208.
3. SCHREIBER, F. 1934. Zur Genetik der weissen Samenfarbe bei *Phaseolus vulgaris*. — Der Züchter, VI, 53—61.
4. SURFACE, FR. M. 1916. A Note on the Inheritance of Eye Pattern in Beans and Its Relation to Type of Vine. — Amer. Nat., L, 577—586.
5. TSCHERMAK, E. v. 1912. Bastardierungsversuche an Levkojen, Erbsen und Bohnen mit Rücksicht auf die Faktorenlehre. — Z. f. ind. Abst. u. Vererbgl., VII, 81—234.

ZUR GENETIK VON PHASEOLUS VULGARIS XVII—XVIII

VON *HERBERT LAMPRECHT*
SAATZUCHTANSTALT WEIBULLSHOLM, LANDSKRONA

(With a Summary in English)

XVII. ZWEI NEUE GENE FÜR ABZEICHEN AUF DER TESTA, Punc UND Mip, SOWIE ÜBER DIE WIRKUNG VON V UND Inh.

ALS Abzeichen auf der Testa von *Phaseolus vulgaris* werden vom Hilumrand oder dessen unmittelbarer Nähe ausgehende, lokalisierte Zeichnungen aufgefasst. Von solchen sind bisher vier auf ihre Vererbung hin untersucht worden. Es sind dies Carunculastrich, Mikropylenstreifen, Corona und Margo.

Der *Carunculastrich* bildet eine Fortsetzung der Spitze der Caruncula nach aussen. Er stellt einen farbigen Strich in dunklerer Farbe als die Testa dar und ist an seinem Ende gewöhnlich deutlich gabelförmig gespalten (vgl. Fig. 1). Dieses Abzeichen wird durch das dominante Gen *Ca* bedingt (LAMPRECHT, 1932 b). Die Farbe des Carunculastriches liegt in den Pallisadenzellen.

Der *Mikropylenstreifen* bildet einen etwa 0,5—0,7 mm breiten, gewöhnlich graulichen Streifen, der, wie der Namen andeutet, auf der Mikropylenseite gleich ausserhalb des Hilumrandes seinen Ausgang nimmt und bis zum oder über das schmale Ende des Samens fortsetzt (vgl. Fig. 1). Er stellt eine rezessive Eigenschaft dar und wird durch das Gen *mi* bedingt. Er scheint nur bei Anwesenheit von *J* realisiert werden zu können (LAMPRECHT, 1932 b).

Die *Corona* bildet einen farbigen, den Hilumrand aussen umfassenden Ring, der an der Stelle der Caruncula, soweit bisher beobachtet, stets unterbrochen ist. Die Corona kann vom Hilumrand zuweilen durch einen feinen helleren Rand getrennt sein oder sie schliesst — häufiger — direkt an den Hilumrand an (vgl. Fig. 2). Die Farbe der Corona ist von der genotypischen Konstitution für die Testafarbe abhängig. Gewisse Testafarben scheinen also nur mit bestimmter Coronafarbe vorkommen zu können. Die Ausbildung der Corona wird durch das rezessive Gen *cor* bedingt. *Cor cor*-Samen haben schwach angedeutete Corona (LAMPRECHT, 1934).

Die *Margo* schliesslich bildet einen breiten, farbigen Rand, eine sog. Bräme aussen rund um Corona bzw. Hilumrand. Die Abgrenzung der Margo gegen die Farbe der übrigen Testa ist, zum Unterschied gegen die vorher genannten drei Abzeichen, nicht scharf sondern diffus (vgl.

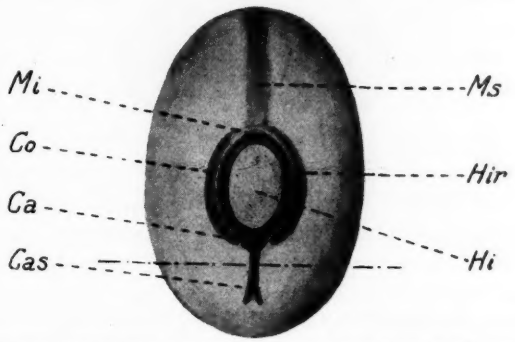


Fig. 1. Abbildung einer Bohne, verschiedene Elemente bzw. Zeichnungen der Testa veranschaulichend. Hi = Hilum, Hir = Hilumrand, Ca = Caruncula, Cas = Caruncula-strich, Mi = Mikropyle, Ms = Mikropylenstreifen, Co = Corona.

Fig. 3). Die Ausbildung der Margo wird durch das rezessive Gen *mar* verursacht. Die Margo kann in ganz verschiedenen Farben auftreten. Eine Reihe von solchen Fällen liegen nunmehr eingehend genetisch analysiert vor (LAMPRECHT, 1933 und 1939). Es zeigte sich, dass die

Fig. 2. Drei Samen der Testafarbe Geschwefeltes Weiss. Linker Same ohne Corona, *CorCor*, rechter Same mit starker Corona, *corcor*, mittlerer Same mit schwach angedeutete Corona, *Corcor*.

Margo nur bei Rezessivität im Gen *J* ausgebildet werden kann. Die Margo erscheint nämlich stets in der Testafarbe, die durch Umwandlung von *j* in *J* erhalten wird. Speckweisse Samen, *P G*, bekommen also durch *mar* eine Margo in der *P G J* entsprechenden Farbe, d. i. Maisgelb; und Gleiches gilt in allen bisher studierten Fällen. Das Gen

mar bedingt also nichts anderes, als lokale Umwandlung von Rezessivität in Dominanz, nämlich von *j* in *J*.

Im folgenden werden zwei weitere Abzeichen der Testa hinsichtlich ihres Erbganges untersucht werden. Es sind dies die vom Hilumrand ausgehende Punktierung und die sog. Mikropylarpunkte.

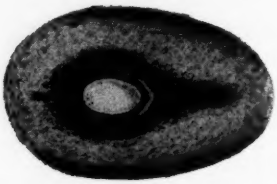


Fig. 3. Same der Testafarbe Veilchenartig Weiss mit hell Bister Hilumrand und Havannabrauner Bräme (Margo) um letzteren.

DIE VERERBUNG DER PUNKTIERUNG DER TESTA.

Bei Punktierung beobachtet man auf der Samenschale zerstreut liegende Punkte in dunklerer, aber im übrigen ähnlicher Farbe wie die Testa. Ist z. B. die Testa Rhamninbraun, so sind die Punkte Dkl. Rhamninbraun bis Mineralbraun. Die Form der Punkte ist eine unregelmässige. Ihre Verteilung auf der Testa geht aus Fig. 4 hervor. Wie aus dieser ersichtlich, liegen die Punkte in der Nähe des Hilums dichter und auf der gegenüberliegenden Seite gewahrt man in der Regel nur ganz vereinzelte solche. Mitunter sind die Punkte überhaupt nur in der Nähe des Hilums wahrzunehmen. Bei okulärer Besichtigung machen diese Punkte den Eindruck von kleinen Grübchen. Eine nähere Untersuchung zeigt indessen, dass die Oberfläche keine Einsenkungen aufweist. Diese Erscheinung beruht zweifellos auf Lichtbrechung; denn ein Schnitt durch die Samenschale (vgl. Fig. 5)

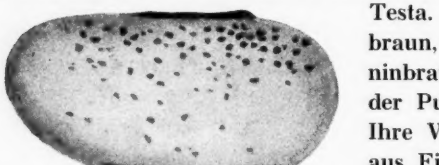


Fig. 4. Same mit punktierter Testa, Formel: *puncpunc*.

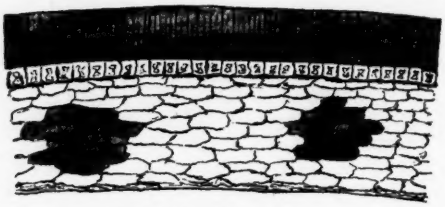


Fig. 5. Querschnitt durch die Testa eines punktierten Samens. Die Punkte repräsentieren sich hier als dunkler gefärbte Zelleninseln in der Parenchymschicht.

zeigt, dass diese Punkte auf farbige Zelleninseln in der tiefer gelegenen Parenchymschicht der Samenschale zurückzuführen sind.

Punktierte Samen wurden von mir zuerst auf einer Pflanze angetroffen, die aus einer spontanen Kreuzung in der Sorte Flageolet Wachs stammte. Die Nachkommen dieser Pflanze waren konstant und wurden als Linie 157 weitergeführt. Die Testafarbe dieser Samen ist Rhamninbraun. Mehrere Kreuzungen, ausgeführt zwischen dieser und nicht punktierten Linien zeigten, dass die Punktierung eine rezessive Eigenschaft ist.

Kreuzung Nr. 118 wurde ausgeführt zwischen L. 157 und L. 27. Linie 27 stammt aus der französischen Wachsbohne De Digoin. Ihre Formel hinsichtlich Testafarbe ist *P c J g b v r*. Sie wurde mehrmals genetisch analysiert. F_1 dieser Kreuzung zeigte, wie zu erwarten, Rhamninbraune Samen. Der Farbe Rhamninbraun kommt die Formel *P c J G B v r* zu. Von einer Punktierung war auf diesen nichts wahrzunehmen.

Die zweite Generation zeigte hinsichtlich Punktierung folgende Aufspaltung: 570 ohne Punktierung : 196 mit Punktierung. Es handelte sich offenbar um eine monohybride Spaltung. D/m beträgt hierfür 0,38, also sehr gute Übereinstimmung mit den theoretisch erwarteten Spaltungszahlen anzeigend. Das für die Punktierung verantwortliche Gen belege ich, abgeleitet von der rezessiven Eigenschaft punktiert, mit dem Symbol *punc* (von *punctatus*).

Hinsichtlich Testafarbgenen fand noch Spaltung in *G* : *g* und *B* : *b* statt. In diesen beiden sowie im Genpaar *Punc—punc* ergaben sich zusammen folgende bifaktorielle Spaltungen.

Gefunden:	401 *G B*	: 168 *G b*	: 139 *g B*	: 58 *g b*
Erwartet:	430,87 »	: 143,62 »	: 143,62 »	: 47,87 »
D/m für				
9 : 3 : 3 : 1 =	— 2,17	+ 2,26	— 0,43	+ 1,51

Gefunden:	454 *G Punc*	: 129 *G punc*	: 116 *g Punc*	: 67 *g punc*
Erwartet:	430,87 »	: 143,62 »	: 143,62 »	: 47,87 »
D/m für				
9 : 3 : 3 : 1 =	+ 1,68	— 1,35	— 2,56	+ 2,86

Gefunden:	393 *B Punc*	: 147 *B punc*	: 177 *b Punc*	: 49 *b punc*
Erwartet:	430,87 »	: 143,62 »	: 143,62 »	: 47,87
D/m für				
9 : 3 : 3 : 1 =	— 2,76	+ 0,31	+ 3,09	+ 0,17

Da die letzte Spaltung, in den Genpaaren *B—b* und *Punc—punc*, grössere Abweichungen vom theoretisch erwarteten Verhältnis aufweist, werden noch die auf Grund der monohybriden Spaltungsverhältnisse zu erwartenden Werte mitgeteilt.

Erwartet:	402,1 *B Punc* :	137,9 *B punc* :	168,2 *b Punc* :	56,8 *b punc*
D/m =	— 0,66	+ 0,84	+ 0,81	— 1,31

Nun besteht befriedigende Übereinstimmung. Die drei Gene *G*, *B* und *Punc* sind also wahrscheinlich nicht miteinander gekoppelt.

Mit Hinblick auf die durch das Gen *mar* bedingte lokale Umwandlung von Rezessivität in Dominanz des Gens *j* (vgl. Einleitung) entsteht hier analog die Frage, ob das Gen *punc* selbst für eine bestimmte Färbung der in Frage stehenden Zelleninseln in der Parenchymschicht der Testa verantwortlich ist, oder ob es dort nur die Wirkung gewisser Testafarbgene auslöst. Sicher festgestellt ist, dass die Farbe der Zelleninseln (mit Ausnahme von schwarzer Testa) stets eine dunklere ist als die den Pallisadenschicht. Im vorliegenden Fall ist die Pallisadenschicht Rhamninbraun, *P J G B*, die der Zelleninseln (= Punkte) am nächsten Mineralbraun, *P C J G B*. Es erscheint daher nicht unmöglich, dass das Gen *punc* in den Zelleninseln das Auftreten einer Farbe bedingt, die der genotypischen Konstitution der Testafarbe vermehrt durch die Wirkung von *C* entspricht. Dies würde bedeuten, dass bei Samen mit der Konstitution *C* die Farbe der Zelleninseln gleich der der Pallisaden sein sollte. Weitere Studien werden dies klarlegen.

DIE VERERBUNG DES ABZEICHENS MIKROPYLARPUNKTE.

Samen mit Mikropylarpunkten wurden von mir als Einmischung in einer in Ungarn vermehrten Partie von Flageolet Wachs gefunden. Sie zeigten die Testafarbe Blass Glaucescens, sollten demnach die genotypische Konstitution *P c j g b V r* besitzen. Dies konnte in Kreuzungsversuchen auch bestätigt werden. Im übrigen handelte es sich um eine ziemlich späte, niedrige Brechbohne.

Die Mikropylarpunkte bilden kleine unregelmässige Fleckchen in grünlich blaugrauer Farbe. Sie ist also sehr ähnlich der Testafarbe Blass Glaucescens, nur bedeutend dunkler. Eine anatomische Untersuchung der Testa ergibt, dass die Mikropylarpunkte aus eben so gefärbten Gruppen von Pallisadenzellen bestehen. Wie ihr Namen andeuten soll, nehmen sie ihren Ausgang von der Mikropyle oder deren unmittelbaren Nähe. Ihre Verbreitung auf der Testa ist am besten aus

Fig. 6 ersichtlich. Von der Mikropyle gehen bogenförmig seitlich nach unten divergierend zwei Gruppen von Punkten aus. Die Grösse dieser Punktegruppen variiert ziemlich stark in verschiedenen Linien. Bei gewissen können sich die Punkte über den grösseren Teil der Samenseiten erstrecken, bei anderen bilden sie nur je eine kleine Gruppe seitlich der Mikropyle (vgl. Fig. 6 unten).

Die nach der zuerst angetroffenen Pflanze mit Mikropylarpunkten gebaute Linie 98 zeigte die Punkte in mittlerer Verbreitung. Erst in Kreuzungen sind dann Typen mit verschiedener Ausbreitung erhalten worden. Eine Kreuzung, Nr. 151, wurde ausgeführt zwischen L. 98 und L. 147. Letztere, eine Wachsbohne, stammt aus meiner Kreuzung Nr. 12 und hat die genotypische Konstitution *P c j g b v r*; sie ist demnach in allen Testafarbgenen rezessiv. Die auf F_1 dieser Kreuzung erhaltenen Samen zeigten die gleiche Testafarbe wie L. 98, also auch Mikropylarpunkte. Anscheinend stellten also die Mikropylarpunkte eine dominante Eigenschaft dar. Da die diesbezügliche genotypische Konstitution des anderen Elters, L. 147, indessen nicht sicher bekannt war, konnte erst F_2 hierauf endgültig antworten. Folgende Spaltung ergab sich.

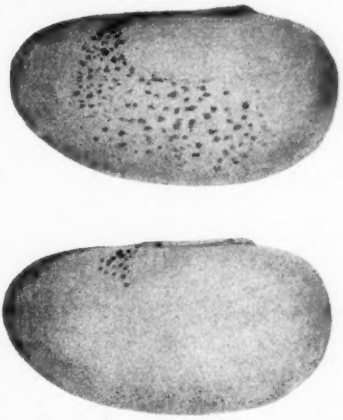


Fig. 6. Zwei Samen mit dem Abzeichen Mikropylarpunkte auf der Testa. Beim oberen Samen ist dieses Merkmal stark, beim unteren schwach ausgebildet.

Gefunden: 473 mit Mikropylarpunkten: 134 ohne Mikropylarpunkten
Erwartet: 455,25 » : 151,75 » »
D/m für 3 : 1 = 1,66.

Die Eigenschaft Mikropylarpunkte ist also dominant und zeigt monohybride Spaltung nach 3 : 1. Das hierfür verantwortliche Gen belege ich mit dem Symbol *Mip*, abgeleitet von der rezessiven Eigenschaft *Micropyle inpunctata* (nicht punktierte Mikropyle).

Linie 98 hat also die Formel *P c j g b V r Mip*, L. 147 *P c j g b v r mip*. Von Interesse ist nun, dass nicht nur Samen mit Testafarbe, im vorliegenden Fall Blass Glaucescens, sondern auch Reinweisse Samen ohne ein dominantes Testafarbgen, wohl aber mit dem Grund *P*, Mikro-

pylarpunkte hatten. Die Farbe dieser war dann nicht Grünlich Blaugrau sondern Blass Gelblich, etwa der Testafarbe Geschwefeltes Weiss nahekommend. Für die beiden Genpaare *V*—*v* und *Mip*—*mip* hat sich ergeben:

Gefunden:	358	*V Mip*: 90	*V mip*: 115	*v Mip*: 44 *v mip*
Erwartet:	341,44	» : 113,81	» : 113,81	» : 37,94 »
D/m für				
9 : 3 : 3 : 1 =	+ 1,35	— 2,48	+ 0,12	+ 1,02

Die gefundenen Spaltungszahlen zeigen befriedigende Übereinstimmung mit den theoretisch erwarteten.

Weiter seien noch die Spaltungszahlen für das Genpaar *Y*—*y* (grüne—gelbe Hülsenfarbe) zusammen mit *Mip*—*mip* und *V*—*v* mitgeteilt.

Gefunden:	373	*Y Mip*: 95	*Y mip*: 100	*y Mip*: 39 *y mip*
Erwartet:	341,44	» 113,81	» : 113,81	» : 37,94 »
D/m für				
9 : 3 : 3 : 1 =	+ 2,58	— 1,96	— 1,44	+ 0,18

Gefunden:	349	*Y V*: 119	*Y v*: 99	*y V*: 40 *y v*
Erwartet:	341,44	» : 113,81	» : 113,81	» : 37,94 »
D/m für				
9 : 3 : 3 : 1 =	+ 0,62	+ 0,54	— 1,54	+ 0,35

Auch diese Zahlen zeigen befriedigende Übereinstimmung mit den erwarteten Verhältnissen, weshalb die Gene *Mip*, *Y* und *V* wahrscheinlich nicht miteinander gekoppelt sind.

Schliesslich sei erwähnt, dass auch die Mikropylarpunkte auf verschiedenen Testafarben in bestimmten, aber verschiedenen Farben auftreten. Auch hier könnte hinsichtlich der Wirkung des Gens *Mip* Analoges gelten, was im vorigen Abschnitt für *punc* und früher für *mar* angeführt worden ist. Das Gen *Mip* könnte in den Punkten, in den diesen entsprechenden Gruppen von Pallisadenzellen, die Wirkung eines Farbgens (z. B. *C*) auslösen, d. h. dieses dort von Rezessivität in Dominanz verwandeln, und so zusammen mit der übrigen genotypischen Konstitution für Testafarbe einen bestimmten Effekt hervorbringen. Hierbei muss auch auf eine eventuelle Beeinflussung der Farbenmodifikationsgene *Vir*, *Och* und *Flav* Rücksicht genommen werden. So könnte z. B. die grünlichblaugraue Farbe der Mikropylarpunkte von L. 98 der Formel *P C j g b V r Vir* entsprechen. Diese bedingt nämlich

Graugrüne Testafarbe. Auch hier werden natürlich erst Spezialstudien klaren Bescheid geben können.

ÜBER DIE WIRKUNG DER GENE V UND Inh.

Das Gen *V* ist seit langem bekannt (vgl. LAMPRECHT, 1932 a und 1935). Es sind drei Allelen bekannt, nämlich *V*, v_{lae} und *v*. Wahrscheinlich besteht noch ein viertes Allel v_{pal} (vgl. LAMPRECHT, 1936). Für die drei Allelen *V*, v_{lae} und *v* sind pleiotrope Effekte bekannt. Das Allel *V* bedingt Bischoffsviolette Blütenfarbe, Rote Stammfarbe und zusammen mit dem Grundgen *P* und *Gri* allein die Testafarbe Blass Glaucescens. Zusammen mit anderen Testafarbgenen verursacht *V* dunklere Farben in blauen—violetten Tönen bis Reinschwarz. Das Allel v_{lae} bedingt die Blütenfarbe Laeliafarbig und die Stammfarbe Rosa. Auf die Testafarbe scheint es keinen Einfluss zu besitzen. Bei Rezessivität in *v* sind die Blüten Weiss, der Stamm rein Grün und die Testa (bei Anwesenheit anderer Farbgene) Reinweiss.

Sehr charakteristisch für die Wirkung von *V* auf die Testafarben ist, dass die Wirkung dieses Gens beim Ausreifen und damit der Färbung der Samen stets zuletzt in Erscheinung tritt. Ein Beispiel möge dies illustrieren. Die Testafarbe Veilchenviolett hat die Formel *P C J V*. Der Formel *P C J v* entspricht die Testafarbe Schamois. Beim Ausreifen der Samen zeigen diese nun stets zuerst die Farbe Schamois, die dann schmutzig Schamois wird, darauf hellvioletten Anflug bekommt und schliesslich dunkler bis zu Dunkel Veilchenviolett wird. Wenn solche Samen nicht gut ausreifen, so kann auch die endgültige Farbe ein schmutzig Braunviolett sein. Diese Umwandlung der Testafarbe geht stets vom Hilumrand aus und verbreitet sich allmählich über die ganze Samenschale. Am besten ist diese Farbe daher stets in der Nähe des Hilums ausgebildet. Genau das Gleiche gilt für alle Testafarben, die *V* in ihrer genotypischen Konstitution enthalten.

Worauf beruht nun dieser durch das Gen *V* bedingte, erst beim Ausreifen eintretende Ausfärbungsprozess? Die anatomische Untersuchung des Testaquerschnittes scheint hier Aufschluss gebenden Bescheid zu liefern. Es zeigt sich, dass die Pallisadenzellen bei allen Testafarben, ausgenommen mit *V*, gleichmässig gefärbt erscheinen. Bei Samen der Formel *P V* enthalten diese Zellen einen grünlich blaugrauen Niederschlag in der Form von feinen Körnchen. Und dasselbe scheint für alle *V* in ihrer Formel enthaltenden Genotypen Gültigkeit zu haben. Ausgenommen hiervon sind natürlich sämtliche in den Grundgenen für

Testafarbe, *P* und *Gri*, Rezessiven, die überhaupt keinen Farbstoff ausbilden können.

Das Gen *V* verursacht demnach eine Fällungsreaktion, die erst bei gewisser Konzentration des Zellsaftes während des Ausreifens einzusetzen beginnt. Dies erklärt auch, weshalb die Ausfärbung der Testa von *V*-Samen in hohem Grade von den zur Zeit des Ausreifens herrschenden Umweltverhältnissen abhängig ist. Ausserdem dürfte diese Erscheinung auch von der übrigen genotypischen Konstitution beeinflusst werden. So konnte ich schon lange beobachten, dass gewisse *V*-Linien gewöhnlich typische dunkle Farbe zeigten, während andere niemals eine solche erreichten, sondern meistens nur die entsprechende Farbe mit *v* in rezessiver Form und etwas Anflug in der erwarteten Farbe hatten. Besonders deutlich war dies hinsichtlich der Testafarben Graulich Indigo, *P c J G B V*, Dkl. Indigo, *P c J g B V*, Ageratumblau, *P c J G b V*, und Eisenhutviolett, *P c J g b V*.

Die vorhin erwähnte Kreuzung Nr. 151 scheint diesbezüglich Aufschluss zu geben. In dieser spalteten, abgesehen von *Y—y* für grüne—gelbe Hülsenfarbe, die beiden Genpaare *V—v* und *Mip—mip*. Das Vorhandensein von dominantem *V* konnte leicht stets an der Blütenfarbe ermittelt werden. Mikropylarpunkte, *Mip* entsprechend, traten nun

F_1: *PP Vv Mip-mip Inhinh*						
Blass Glaucescens mit Mikropylarpunkten	F_2:	48 *V*	36 *Mip*	27 *Inh*	287 256,08 (1,72)	Blass Glaucescens mit Mikropylarpunkten: *P V Mip Inh.*
				9 *inh*	71 85,34 (1,61)	Reinweiss mit Mikropylarpunkten: *P V Mip inh.*
			12 *mip*	9 *Inh*	74 85,34 (1,31)	Blass Glaucescens ohne Mikropylarpunkte: *P V mip Inh.*
				3 *inh*	16 28,45 (2,43)	Reinweiss ohne Mikropylarpunkte: *P V mip inh.*
		16 *v*	12 *Mip*	9 *Inh*	115 113,81	Reinweiss mit Mikropylarpunkten: *P v Mip (Inh inh).*
				3 *inh*	(0,12)	
			4 *mip*	3 *Inh*	44 37,94	Reinweiss ohne Mikropylarpunkte: *P v mip (Inh inh).*
				1 *inh*	(1,02)	

Die Aufspaltung des Bastards PP Vv Mipmip Inhinh in F_2 von Kreuzung Nr. 151.
Vor den Farbenbezeichnungen steht die Anzahl gefundener Individuen, darunter die erwartete Anzahl und in Klammern der Wert für D/m.

sowohl auf *V*- wie auf *v*-Samen auf. Im ersten Fall waren sie von grünlich-blaugrauer Farbe, im letzteren von hellgelber. Die *V*-Pflanzen konnten aber überdies noch in zwei Gruppen eingeteilt werden, eine mit der Testafarbe Blass Glaucescens und eine mit Reinweisser Testa. Beide hatten die Mikropylarpunkte in gleicher Farbe ausgebildet. Das beigegebene Spaltungsschema zeigt die hierfür erhaltenen Zahlen.

Wie ersichtlich wurde in dieser Kreuzung innerhalb der *V*-Pflanzen eine deutlich monohybride Spaltung nach 3 mit Blass Glaucescens : 1 Reinweiss gefunden. Für die letztere Gruppe ist also anzunehmen, dass die sonst beim Ausreifen der Samen durch *V* bedingte Ausfällung von grünlich-blaugrauen Körner im Zellsaft der Pallisadenzellen durch das Vorhandensein eines rezessiven Gens verhindert worden ist. Das hierfür verantwortliche Gen will ich, abgeleitet von *inhibeo* = hemmen, mit dem Symbol *Inh* bezeichnen. Eine sichere Klassifikation in bezug auf dieses Gen wird allerdings nicht stets möglich sein. Nur in Kreuzungen mit einigermassen gleichzeitigem Ausreifen werden mit dem theoretisch erwarteten Verhältnis nahe übereinstimmende Werte erhältlich sein. Bei auch hinsichtlich Reifezeit stärker spaltenden Kreuzungen wird die Spaltung im Genpaar *Inh—inh* durch den ähnlich wirkenden Einfluss der Umweltverhältnisse die Grenzen verwischen. — Zu ermitteln verbleibt ob *inh* bei allen Testafarben mit *V* die Wirkung dieses Gens vollständig inhibiert oder nur stark abschwächt. Letzteres wurde wiederholt beobachtet, ohne dass jedoch genetische Analysen ausgeführt worden sind.

SUMMARY.

1. The inheritance of two new points of different colour on the seed coat of *Phaseolus vulgaris* is studied.

2. One of these, the character dotting, is caused by coloured islands of cells in the parenchymal layer of the seed coat (Figs. 4 and 5). This character is singly recessive and corresponds to the new gene pair *Punc—punc*.

3. The second character is the so-called micropyle points. The development of these coloured points always begin in the neighbourhood of the micropyle (see Fig. 6). Micropyle points are a dominant character, which correspond to the new gene pair *Mip—mip* (derived from *micropyle inpunctata*).

4. The effect of the gene *V*, causing the seed coat colour Pale Glaucescens, has been closely studied. It may be mentioned that this

gene causes the precipitation of dark glaucescent fine grains in the pallisade cells, which form the epidermis.

5. A new gene *Inh* (derived from *inhibeo*) has been found, which in its recessive condition, *inh*, prevents the precipitation of glaucescent grains, caused by *V*.

XVIII. ÜBER MATTE SAMENSCHALE UND IHRE VERERBUNG.

Nicht alle Farben der Samen von *Phaseolus vulgaris* zeigen gleich hohen Glanz. So sind z. B. Farben ohne das dominante Gen *J* gewöhn-

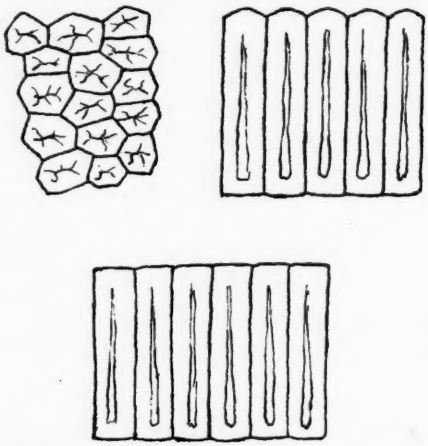


Fig. 7. Oben, links: Flächenansicht der Pallisadenzellen der matten Testa (*aspasp*), rechts: Längsschnitt durch dieselben. Man beachte die Kuppenbildung am oberen (äusseren) Ende. Unten: Längsschnitt durch Pallisadenzellen der normalen, glänzenden Testa (ohne Kuppenbildung).

lich etwas bis deutlich weniger glänzend als solche mit *J*. Stark glänzend ist z. B. Rohseidengelb, *P J*, Schamois, *P C J*, Bister, *P C J G* usw. Weniger stark glänzend bis matt sind Veilchenartig Weiss, *P B*, Ambraweiss, *P C B* usw. Die Struktur der äussersten Zellenschicht solcher mehr oder weniger glänzender Typen zeigt indessen kaum welche sicheren Unterschiede. Die Mattigkeit des im folgenden zu besprechenden Typus ist von diesen stark verschieden, sowohl bei okulärer Betrachtung wie bei anatomischer Untersuchung.

Stark matte Samen wurden von mir aus dem Keniagebiet in Afrika erhalten. Eine Linie dieser mit schwarzer Testafarbe wurde unter Nr. 61

weitergebaut. Die Oberfläche der Samen von L. 61 erscheint typisch sammtartig matt. Bei etwa 20-facher Lupenvergrösserung erscheint die Oberfläche deutlich chagriniert, also körnig. Ein Schnitt durch die Testa gibt diesbezüglich Bescheid und zeigt einen markanten Unterschied gegenüber dem normalen glatten Typus. Wie Fig. 7 zeigt, sind die äusseren Enden der Pallisadenzellen durch eine Kuppe abgeschlossen. Hierdurch wird sowohl die Körnelung der Oberfläche bedingt wie auch im Zusammenhang mit der dadurch verursachten ungleichen Reflexion des Lichtes das sammtartig matte Aussehen. Auch sind die Risse auf den Kuppen stärker zutagetretend als auf den sonst flachen Zellenenden.

Mit Linie 61 wurden mehrere Kreuzungen ausgeführt. Zu Kr. Nr. 148 wurde als zweiter Elter Linie 147 verwendet, die nur in den beiden Grundgenen *P* und *Gri* dominant, in allen Testafarbgenen aber rezessiv ist. Die auf F_1 erhaltenen Samen zeigten glatte Oberfläche, die Mattigkeit, die Rauheit der Testa scheint demnach ein rezessives Merkmal darzustellen. In der zweiten Generation wurden insgesamt 943 Pflanzen untersucht, die folgendermassen spalteten:

Gefunden: 672 mit glatter Testa : 271 mit matter Testa
Erwartet: 707,25 » » » : 235,75 » » »
D/m für 3 : 1 = 2,65.

Die Übereinstimmung zwischen gefundenen und bei Monohybridie theoretisch erwartetem Spaltungsverhältnis ist keine gerade gute, aber zweifellos noch als monohybrid aufzufassen. Das für die Spaltung in glatte : matte (rauhe) Testa verantwortliche Genpaar belege ich mit den Symbolen *Asp—asp*, abgeleitet von der rezessiven Eigenschaft *asper* = rauh.

SUMMARY.

1. Seeds of *Phaseolus vulgaris* with a rough, lustreless surface were received from Kenya, Africa.

2. The roughness of the seed coat is caused by elevations (like cupolas) of the ends of the pallisade cells, which form the epidermis (Fig. 1).

3. The character of roughness of the seed coat is singly recessive, corresponding to the gene pair *Asp—asp* (derived from *asper* = rough).

ZITIERTE LITERATUR.

1. LAMPRECHT, H. 1932 a. Beiträge zur Genetik von *Phaseolus vulgaris*. Zur Vererbung der Testafarbe. — Hereditas, XVI, 169—211.
2. — 1932 b. Zur Genetik von *Phaseolus vulgaris*, III. Zweiter Beitrag zur Vererbung der Testafarbe. — Hereditas, XVII, 1—20.
3. — 1933. Zur Genetik von *Phaseolus vulgaris*, VI. Vierter Beitrag zur Vererbung der Testafarbe. — Hereditas, XVIII, 249—316.
4. — 1934. Zur Genetik von *Phaseolus vulgaris*, VII. Zwei weitere Gene für Sameneigenschaften, *Cor* und *Fast*. — Hereditas, XIX, 163—176.
5. — 1935. Zur Genetik von *Phaseolus vulgaris*, XII. Über die Vererbung der Blüten- und Stammfarbe. — Hereditas, XXI, 129—166.
6. — 1936. Zur Genetik von *Phaseolus vulgaris*, XIII. Ein neues Grundgen für Testafarben, ein weiteres Testafarbgen sowie etwas über Blütenfarben. — Hereditas, XXII, 241—268.
7. — 1939. Lokale Umwandlung von Rezessivität in Dominanz durch die Wirkung eines besonderen Gens bei *Phaseolus vulgaris*. — Z. f. ind. Abst. u. Vererbgl. LXXVII, 186—194.

INHALT.

XVII. Zwei neue Gene für Abzeichen auf der Testa, *Punc* und *Mip*, sowie über die Wirkung von *V* und *Inh* 292
Die Vererbung der Punktierung der Testa 294
Die Vererbung des Abzeichens Mikropylarpunkte 296
Über die Wirkung der Gene *V* und *Inh* 299
Summary 301
XVIII. Über matte Samenschale und ihre Vererbung 302
Summary 303
Zitierte Literatur 304

CYTOLOGY OF AGROPYRON JUNCEUM, A. REPENS AND THEIR SPONTANEOUS HYBRIDS

BY *G. ÖSTERGREN*
INSTITUTE OF GENETICS, LUND, SWEDEN

THE material for this study was obtained from natural localities in South Sweden. The root tips and anthers were fixed in chrome-acetic-formalin. The preparations were stained with gentian violet.

Somatic cytology. — *Agropyron junceum* (L.) P. B. was found to have $2n = 28$. Material from five localities was studied. Counts were made on 9 plants, 7 determinations were exact (3 plates), and 2 approximate.

The chromosome number $2n = 28$ was previously observed in this species by PETO (1930), SIMONET (1934, 1935 a and b), PARDI (1937), and SIMONET and GUINOCHET (1938). It was found that *A. junceum* consists of two subspecies, an atlantic form with $2n = 28$, and a Mediterranean form with $2n = 42$ (SIMONET, 1935 a and b; PARDI, 1937; SIMONET and GUINOCHET, 1938).

SIMONET (1935 b) observed two secondary constrictions in the somatic chromosome complement of the tetraploid type. In 1934 he observed none, his material was then from another locality. PARDI (1937) observed none.

The present writer observed no secondary constrictions in most plates, but in some one such constriction was observed, and in a few two. The observations differed in this respect even between cells belonging to the same plant.

It seems probable to me that the contradictory statements as to the number of secondary constrictions are due to the use of inferior fixatives, and that their actual number may be greater than ever observed. It was pointed out by FLOVIK (1938) that chrome-acetic-formalin fixatives are poorly suited for the study of chromosome morphology, although they are good for the determination of chromosome numbers (l. c. pp. 270—271, 278, and Figs. 6—7).

As pointed out by HEITZ (1931), there is a close relation between secondary constrictions and nucleoli. Although I am not convinced

that all secondary constrictions must be nucleolar organizers, and that all nucleoli are organized by constrictions, it is of interest in this connexion to determine the maximum number of nucleoli. In numerous nuclei in the tetrad cells two nucleoli were found. At interphase four nucleoli were clearly observed in a few cells. As the chromosomes are double at interphase it is not certain that this number really proves the existence of four nucleolar organizers in the gametic chromosome set. That there are at least two is rather certain, however.

Agropyron repens (L.) P. B. (4 clones from 3 localities) was found to have $2n = 42$. The determinations are approximate. There are no reasons, however, to believe that the plants are aneuploids.

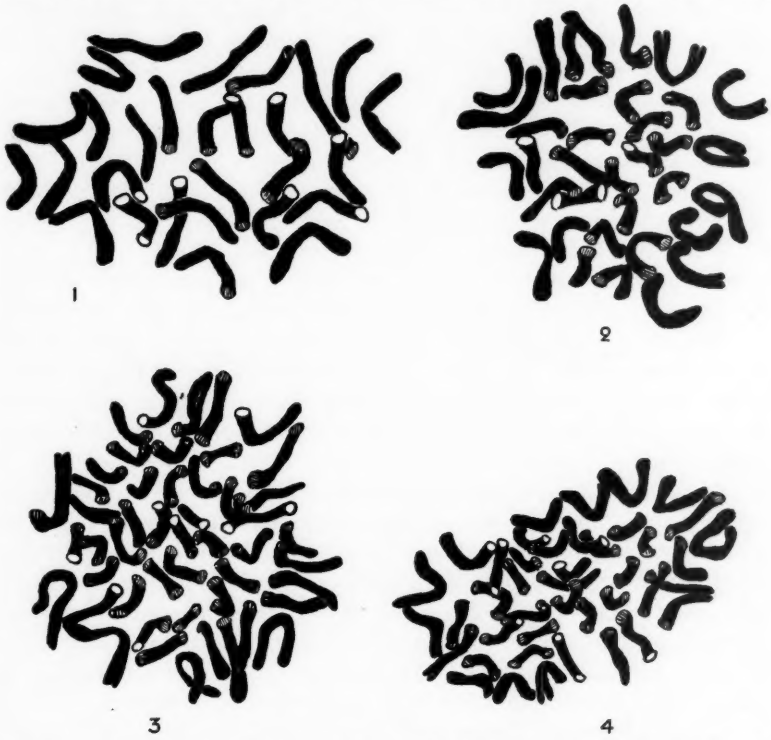
This chromosome number has been reported also by STOLZE (1925), MOWERY (1929), PETO (1929 and 1930), AVDULOW (1931), SIMONET (1935 a) and NIELSEN and HUMPHREY (1937). PETO (1930) found $2n = 35$ in material from U.S.S.R. AVDULOW found $2n = 28$ in a material which probably is also from U.S.S.R., as this author is a Russian.

Hybrids between *A. junceum* and *A. repens* from five Swedish localities were found to have $2n = 35$. As there are apparent morphological differences between some plants collected in the same locality, the number of different clones with this number is 7 (4 determined exactly and 3 approximately). A number of plants from Falsterbo (province of Skåne, South Sweden) had $2n = 49$. As they are morphologically very much alike, they no doubt belong to the same clone. All observations did not give exactly 49 chromosomes, but the variation may be ascribed to the difficulties of making exact counts. These hybrids are then pentaploid and heptaploid, as the basic number of the genus is 7.

35 is the chromosome number to be expected in a hybrid between parents with 28 and 42 chromosomes. This chromosome number was found in the similar hybrids, *A. junceum* (L.) P. B. $\times$ *A. littoreum* (SCH.) ROUY (SIMONET, 1934) and *A. junceum* (L.) P. B. $\times$ *A. littorale* (HOST) DUM. (SIMONET, 1935 a). The hybrid with $2n = 49$ is supposed to contain two gametic chromosome sets from *A. junceum* and one from *A. repens* ($14 + 14 + 21$). (Origin discussed below.) This chromosome number has been observed previously in *Agropyron* in the hybrid between *A. repens* (L.) P. B. ($2n = 42$) and *A. campestre* G. G. ($2n = 56$) (SIMONET, 1935 a).

The chromosomes of *A. junceum* seem to be largest, those of *A. repens* smallest, and those of the hybrids intermediate (Figs. 1—4

and 5—6). It might be suggested that a higher chromosome number causes a smaller chromosome size. Evidence against such an assumption is the fact that PARDI (1937) has found the chromosome size of the tetraploid and the hexaploid types of *A. junceum* to be the same (illustrated with drawings and photographs). The differences in my



Figs. 1—4. Somatic metaphases. — Fig. 1. *Agropyron junceum*, $2n = 28$; Fig. 2, *A. junceum* × *repens*, $2n = 35$; Fig. 3, *A. junceum* × *repens*, $2n = 49$; Fig. 4, *A. repens*, $2n = 42$. — × 2500.

case are probably due to genotypic differences between the two species. (It is known that chromosome size may be genotypically controlled, e. g. DARLINGTON, 1937, pp. 52—60.)

Fertility. — 8 individuals of *A. junceum* were studied as to pollen fertility. 4 of them had a percentage of good pollen of 90—100, 3 had 80—90, and one 70—80. The one with 70—80 % had 107 bad grains in a total of 388 grains. Only one pollen test was made on each indi-

vidual. The tests were made with free pollen and not by crushing the anthers. 3 individuals of *A. repens* were found to have 90—100 % good pollen.

Seed fertility in *A junceum* was studied on spikes gathered from a natural population. The two lowermost flowers in each spikelet were studied. 50 flowers were sterile in a total of 147. RAUNKIAER (1927, p. 344) found 54 % sterility in two hundred spikes of *A. repens*.

Three of the pentaploid hybrids were studied as to pollen fertility. In more than 4000 pollen grains of these plants 3 morphologically good grains were found. The others were quite empty and shrivelled. In the heptaploid hybrid 5 good grains were found in more than 1000. Similar results were obtained in other preparations of this hybrid, and in a preparation made of it in the preceding summer (1938) (grains not counted). The anthers of the hybrids do not dehisce.

No seeds were found in 388 spikelets of the pentaploid hybrids, and in 1496 spikelets of the heptaploid hybrid, after open flowering.

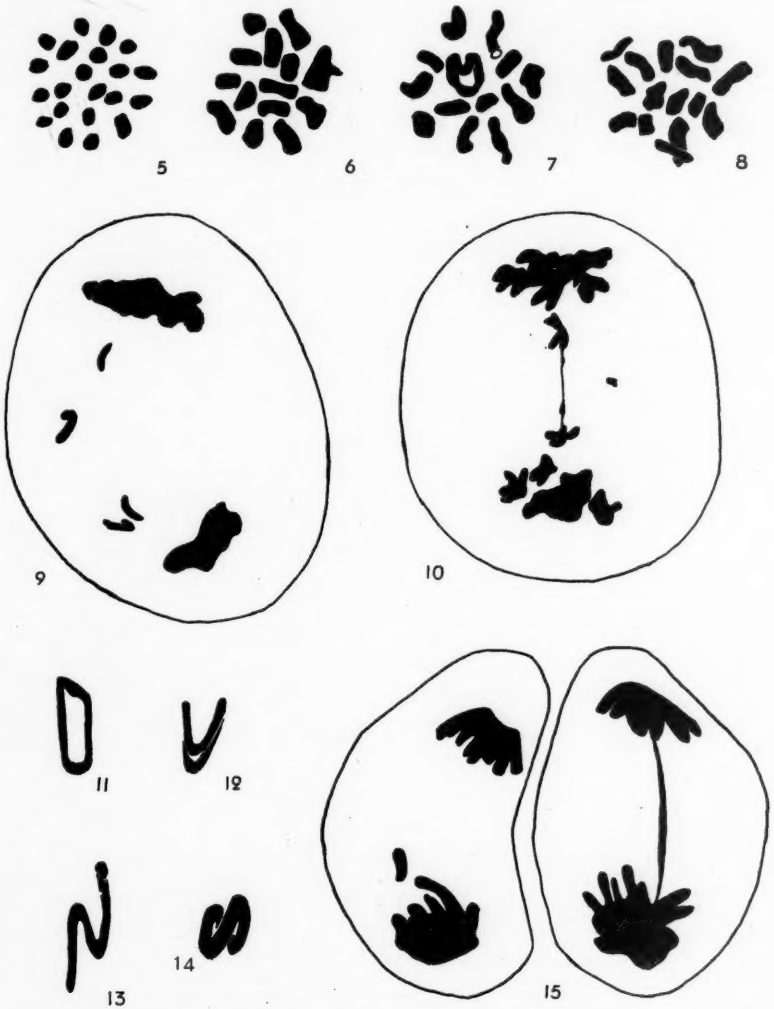
Meiosis in the pure species. — First metaphase in the pure species gives, when regular, 14_{II} in *A. junceum* and 21_{II} in *A. repens* (Figs. 5—6). I have seen no previous statements as to meiosis in *A. junceum*. 21_{II} were found in *A repens* by STOLZE (1925), MOWERY (1929), and PETO (1930).

I also found some irregularities in the two species. Four individuals of *A. junceum* were studied, and all of them were found to be heterozygous for at least one inversion. This was shown by the simultaneous occurrence at first anaphase of a chromosome bridge and a fragment (Fig. 10). In some cases bridges were observed which were not accompanied by fragments. They may have been caused by a delayed chiasma separation. The frequency of inversion bridges is rather low, being estimated at 1—2 per cent approximately.

Univalents were observed in all the four individuals. At metaphase they are distributed at random in the cell or lying at the periphery of the plate (Fig. 8). At anaphase they often lag and divide (Fig. 9). The frequency of cells with univalents was estimated at 10 per cent approximately.

Quadrivalents were observed in three of the individuals. The reason why no quadrivalents were found in the fourth individual may be due to the fact that the material studied of this individual was rather small, or it may be due to constitutional differences.

Seven individuals of *A. repens* were studied. Inversion bridges at first anaphase were found in none, but the material studied of this



Figs. 1—15. Meiosis in the pure species. — Figs. 5—8, first metaphases; Fig. 5, *A. repens*, 21_{II}; Figs. 6—8, *A. junceum*; Fig. 6, 14_{II}; Fig. 7, $12_{II} + 1_{IV}$; Fig. 8, $13_{II} + 2_{I}$; Figs. 9—14, *A. junceum*; Figs. 9—10, first anaphase; Fig. 9, two dividing univalents; Fig. 10, a chromosome bridge and a fragment; Figs. 11—14, quadrivalents drawn from different first metaphases; Fig. 15, *A. repens*, second anaphase with chromosome bridge. — × 2200.

stage was rather small. In acetocarmine preparations of two individuals second anaphase bridges were observed. At the second division a bridge can scarcely be due to a delayed chiasma separation, and I can imagine no other cause than a dicentric chromatid, which may be the result of dyscentric hybridity. These bridges were not, however, accompanied by clear fragments.

Univalents were present in all the *repens* plants studied. Quadrivalents were plainly observed in two individuals, and probably in four others.

It is not possible from these observations of quadrivalents in the two species to state with certainty whether they are caused by segmental interchange or autopolyploidy, which may be partial or complete. I feel inclined, however, to ascribe their occurrence to structural hybridity.

Nor can it be said with certainty that the inversion bridges are due to structural hybridity in the usual sense. They may arise from occasional pairing between chromosomes belonging to different genomes in an allopolyploid, if these genomes differ by inversions. The univalents also may indicate structural hybridity, but that, too, is not certain.

Univalents and quadrivalents were found in the hexaploid species *A. glaucum* by PETO (1936). The quadrivalents in this species were supposed to be caused by segmental interchange (l. c. p. 205). In *A. elongatum* ($2n = 70$) he observed many higher configurations, even octavalents.

If *A. junceum* and *A. repens* are cross-fertilisers, which is probable, judging from their open flowering, the meiotic irregularities found in them might be connected with this mode of reproduction. Structural hybridity seems to be rather common in cross-fertilising plants (MÜNTZING, 1939).

Another fact which might have some influence in this relation is the pronounced vegetative propagation of the species in question. As pointed out by DARLINGTON (1937, p. 271), the hybridity equilibrium in regard to inversions is highest in plants that are largely propagated by asexual means.

For further details on structural hybridity in species and hybrids, see DARLINGTON (1937) and MÜNTZING (1939).

Meiosis in the hybrids. — Meiosis in four pentaploid hybrids was studied. The following frequencies of bivalents and univalents were found.

Univalents	Bivalents	Number of cells
9	13	3
11	12	4
13	11	5
17	9	1
Average 11,8	11,6	Total 13

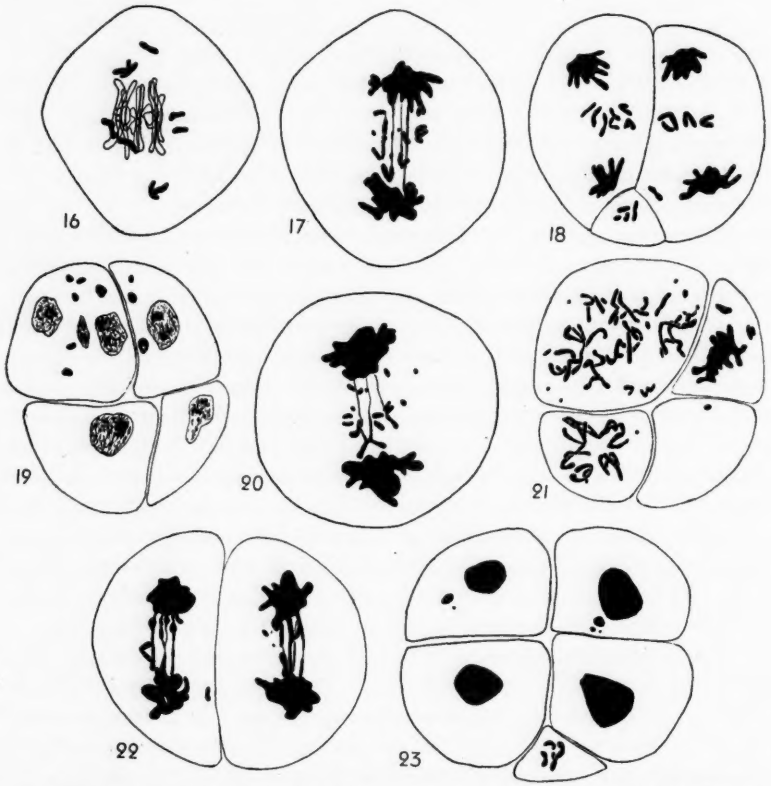
Five cells were studied in one individual, and four cells in each of two others. Judging from the appearance of numerous other cells, the amount of pairing was about the same in them. This is also true of the fourth individual, in which no cell was analysed. In aceto-carmine preparations some possible trivalents were seen. — In one cell in an acetocarmine preparation there seemed to be 35 univalents distributed at random in the cell. The neighbouring cells all showed an amount of pairing of the same order as that recorded in the table.

At first anaphase there often occurs chromosome bridges and fragments, and even more than one bridge in the same cell (Fig. 17). Undoubtedly this hybrid is heterozygous for some inversions. There may have occurred more bridges in one of the individuals than in the others, but I cannot say if the difference is certain. Bridges and fragments were observed at the second division too. Many univalents often lagged and divided at first anaphase. In Fig. 18 there are three cells at second division. The small cell has probably been formed by some univalents. This figure also shows lagging univalents. The tetrad cells often contained extra nuclei and chromatin fragments (Fig. 19). These are probably formed by univalents and acentric fragments.

Thus, it is evident that the hybrid studied is not only a numerical hybrid, but also a structural one. The structural hybridity is indicated also by a reduced chiasma frequency in the pairing chromosomes. It could be seen at once that the proportion of rod-shaped bivalents is much higher in the hybrid than in its parents. The simultaneous occurrence of structural and numerical hybridity is probably the reason why the hybrid is so extremely sterile.

Although the amount of pairing does not deviate very much from that expected on the assumption that the two species have two genomes in common, such a thing cannot, of course, be considered established by the present observations. The pairing might also be due chiefly to autosyndesis, or all the five genomes may be more or less homologous to each other. Assuming, however, that the genotype of the hybrid does not cause a reduced pairing (e. g. by special genes reducing

chiasma frequency), one conclusion can be drawn from the pairing found, viz. that both the species are not completely autopolyploid. There are also other reasons indicating that *A. junceum* is not completely autopolyploid (ÖSTERGREN, 1940 b).



Figs. 16—23. Meiosis in the hybrids. — Figs. 16—19, the pentaploid hybrid; Fig. 16, first metaphase, $13_{II} + 9_{I}$; Fig. 17, first anaphase with bridges; Fig. 18, second anaphase with lagging univalents, and an extra cell with a few chromosomes; Fig. 19, tetrad. — Figs. 20—23, the heptaploid hybrid; Fig. 20, first anaphase, see text; Fig. 21, a supposed second metaphase after a multipolar first division spindle; Fig. 22, second anaphase with chromatin bridges; Fig. 23, tetrad with an extra cell. — × 1400.

According to DARLINGTON (1937, p. 218), the pairing in the hybrid studied by SIMONET (1934) is presumably similar to that of pentaploid *Triticum* and *Nicotiana*, four chromosome sets being associated as pairs

and one remaining chiefly unpaired. The amount of pairing should then not differ very much from that found in my pentaploid hybrids. Nothing is mentioned, however, in SIMONET's paper (1934) as to meiosis in his hybrid, and I do not know the reasons for DARLINGTON's presumption.

If we ascribe *A. junceum* and *A. repens* the genome formulae $J_1J_1J_2J_2$ and $R_1R_1R_2R_2R_3R_3$, the formula of the heptaploid hybrid will be $J_1J_1J_2J_2R_1R_2R_3$, according to the assumptions as to its constitution made above. If *A. repens* were a pronounced allopolyploid, and its chromosomes were not homologous with those of *A. junceum*, the three chromosome sets from *repens* would appear as 21 univalents. If *A. repens* is partly autopolyploid or its chromosomes more or less homologous to those of *A. junceum*, the number of univalents will be reduced by autosyndesis and the formation of trivalents (or other multivalents) with the chromosomes of *A. junceum*.

Because of the high chromosome number of the heptaploid hybrid no first metaphase configurations could be completely analysed. The number of univalents lying apart from the equatorial plate was counted in 77 cells and an average of 4,61 was found. The lowest number, 0, was found in 7 cases and the highest, 12, in one case. In 28 first anaphase cells the number of lagging univalents gave an average of 3,93. It is evident that these numbers must be lower than the actual number of univalents. It does not seem very probable, however, that it amounts to 21. Thus it may be safely concluded that one or both of the factors mentioned above is at work.

The number of chromatin bridges at first anaphase varied in 28 cells from 0 to 3, with an average of 1,43 per cell. The number of fragments varied in these cells from 0 to 7, with an average of 1,68. Fig. 20 shows an interesting case. There is one bridge of the common type, and two others, and these two are both attached to the same centromere. This centromere also carries two normal chromatid arms, as expected under such circumstances. This configuration has probably arisen from dyscentric hybridity in a trivalent. It may also have arisen from a bivalent, but then we should have expected the two bridges to lie more close together. This figure shows dividing univalents and fragments too.

Chromatin fragments are common also at interphase, second metaphase, second anaphase and telophase and in the tetrads. At second anaphase, too, there are often chromatin bridges. The number of cells resulting from the first division is in most cases two, but some excep-

tions were found. Fig. 21 is interpreted as a second division after a multipolar first division spindle. In the lower left cell there are 6 chromosomes. The lower right cell is supposed to have been damaged.

The number of cells in the tetrads is usually 4, but a few cases with 5 were found. In Fig. 23 there are four chromosomes in a supposed anaphase position in the small cell, two in an upper level and two in a lower one. This extra cell has probably been formed by two univalents.

Discussion. — If the heptaploid hybrid contains two gametic chromosome sets from *A. junceum* and one from *A. repens*, as assumed above, it should be expected to resemble *A. junceum* more closely in morphology than the pentaploid hybrids. This is also the case with respect to many characters (ÖSTERGREN, 1940 a; it has for certain reasons been considered appropriate to treat the morphology of these plants in a special paper).

There are two ways in which this heptaploid hybrid may have arisen. The first is the production of an unreduced gamete in a pentaploid hybrid and the fertilisation of this gamete with a normal one from *A. junceum*. There are numerous cases known of non-reduction in hybrids (see, e. g., the table by DARLINGTON, 1937, p. 417). The second possibility is the production of an unreduced gamete in the pure species *A. junceum* and the fertilisation of this with a normal one from *A. repens*. Unreduced gametes in pure species have been found in crosses between *Phleum* species by NORDENSKIÖLD (1937). In a certain cross it was even found that the only gametes functioning on the female side were the unreduced and the doubly unreduced ones (l. c. pp. 306—309). Then the chromosome numbers of the gametes functioning were more alike than they would have been if the reduced gametes had functioned. MÜNTZING (1936) considers such cases to be due to the viability of a zygote being greater the more alike the chromosome numbers are of the gametes giving rise to it. He also gives some other examples (l. c. pp. 326—334). Such a thing might have some influence in my case, too, as the difference between 28 and 21 is relatively smaller than that between 21 and 14.

If *A. junceum* can produce unreduced gametes, this fact might be of interest in the discussion of the origin of the hexaploid subspecies found by SIMONET (1935 a and b). If such an unreduced gamete was fertilised by a normal one, the result would be just a hexaploid individual. It is suggested by SIMONET and GUINOCHET (1938) that the hexaploid type might in some way have arisen from the tetraploid one. I believe, however, that the tetraploid type is an allopolyploid rather

than an autopolyploid. In such a case it could not at once give rise to a fertile and constant hexaploid race, but such a race might, of course, arise in later generations.

The discovery that the hybrid between *A. junceum* and *A. repens* consists of two caryologically different types contributes to the explanation of the variation for which this hybrid is well-known. The variation is, however, caused by other circumstances as well (see ÖSTERGREN, 1940 a).

SUMMARY.

Agropyron junceum has 2n = 28, and *Agropyron repens* 2n = 42, as found also by previous workers. Seven spontaneous hybrids (from South Sweden) between *A. junceum* and *A. repens* were found to have 2n = 35, one hybrid had 2n = 49. The hybrids are highly sterile. Meiosis in the pure species was not always regular, inversion bridges, univalents, and quadrivalents were found. 13 first metaphases in the pentaploid hybrids gave an average of $11{,}6_{II} + 11{,}8_{I}$. In the heptaploid hybrid the chromosomes of *A. repens* are supposed to pair autosyndetically or as trivalents with the *junceum*-chromosomes, to some extent. Both hybrids are heterozygous for inversions. It is assumed that the heptaploid hybrid has arisen from the back-cross of a pentaploid hybrid with *A. junceum* or from the fertilisation of an unreduced gamete of *A. junceum* with a normal one from *A. repens*.

LITERATURE CITED.

1. AVDULOW, N. P. 1931. Karyo-systematische Untersuchung der Familie Gramineen. — Bull. of Appl. Bot. of Gen. and Plant-breed. Suppl. 43, pp. 1—428.
2. DARLINGTON, C. D. 1937. Recent advances in cytology. 2nd ed. — London.
3. FLOVIK, K. 1938. Cytological studies of arctic grasses. — Hereditas XXIV, pp. 263—376.
4. HEITZ, E. 1931. Die Ursache der gesetzmässigen Zahl, Lage, Form und Grösse pflanzlicher Nukleolen. — Planta 12, pp. 775—844.
5. MOWERY, M. 1929. Development of the pollen grain and the embryo sac of *Agropyron repens*. — Bull. of the Torrey Bot. Club 56, pp. 319—324.
6. MÜNTZING, A. 1936. The evolutionary significance of autopolyploidy. — Hereditas XXI, pp. 263—378.
7. — 1939. Chromosomenaberrationen bei Pflanzen und ihre genetische Wirkung. — Zeitschr. f. ind. Abst.- und Vererb.-lehre, LXXVI, pp. 323—350.
8. NIELSEN, E. L. and HUMPHREY, L. M. 1937. Grass studies. I. Chromosome

numbers in certain members of the tribes *Festuceae, Hordeae, Aveneae, Agrostideae, Chlorideae, Phalarideae* and *Tripsaceae*. — Amer. Journ. of Bot. 24, pp. 276—279.

9. NORDENSKIÖLD, H. 1937. Intra- and interspecific hybrids of *Phleum pratense* and *P. alpinum*. — Hereditas XXIII, pp. 304—316.
10. PARDI, L. 1937. Il numero dei cromosomi dell' »*Agropyrum junceum*» P. B. del litorale atlantico e del litorale mediterraneo. — Nuovo Giorn. Bot. Ital., Nuova Serie, XLIV, pp. 645—651.
11. PETO, F. H. 1929. Chromosome numbers in the Agropyrons. — Nature 124, pp. 181—182.
12. — 1930. Cytological studies in the genus *Agropyron*. — Canad. Journ. of Research, Vol. III, p. 428—448.
13. — 1936. Hybridization of *Triticum* and *Agropyron*. II. Cytology of the male parents and F_1 generation. — Canad. Journ. of Research. Sect. C, Vol. XIV, pp. 203—214.
14. RAUNKIAER, C. 1927. Om danske *Agropyrum*-Arter. (Isoreagent-Studier II.) — Botanisk Tidsskrift 39, pp. 329—347.
15. SIMONET, M. 1934. Sur la valeur taxinomique de l'*Agropyrum acutum* ROEHM. et S. — Contrôle cytologique. — Bull. de la Soc. Bot. de France LXXXI, pp. 801—814.
16. — 1935 a. Contribution à l'étude cytologique et génétique de quelques *Agropyrum*. — Compt. Rend. hebd. d. séanc. de l'Acad. des Scienc. 201, pp. 1210—1212.
17. — 1935 b. Observations sur quelques espèces et hybrides d'*Agropyrum*. I. Revision de l'*Agropyrum junceum* (L.) P. B. et de l'*A. elongatum* (HOST) P. B. d'après l'étude cytologique. — Bull. de la Soc. Bot. de France LXXXII, pp. 624—632.
18. SIMONET, M. and GUINOCHET, M. 1938. Observations sur quelques espèces et hybrides d'*Agropyrum*. II. Sur la répartition géographique de l'*Agropyrum junceum* (L.) P. B. — Bull. de la Soc. Bot. de France 85, pp. 175—179.
19. STOLZE, K. V. 1925. Die Chromosomenzahlen der hauptsächlichsten Getreidearten nebst allgemeinen Betrachtungen über Chromosomen, Chromosomenzahl und Chromosomengrösse im Pflanzenreich. — Bibliotheca Genetica 8, pp. 1—71.
20. ÖSTERGREN, G. 1940 a. On the morphology of *Agropyron junceum* (L.) P. B., *A. repens* (L.) P. B. and their spontaneous hybrids. — Botaniska Notiser. (In the press.)
21. — 1940 b. A hybrid between *Triticum turgidum* and *Agropyron junceum*. — Hereditas XXVI.

NOTE ON THE SOMATIC CHROMOSOMES OF SOME COLCHICUM SPECIES

BY *ALBERT LEVAN*
CYTO-GENETIC LABORATORY, SVALÖF, SWEDEN

DUE to their immunity to colchicine and sensitivity to other chemicals with a similar action as colchicine (LEVAN, 1940), the chromosomes of *Colchicum* must be considered to be of special cytological interest. Since extremely few cytological data are published on this genus, I shall in the present paper give a short account of a few observations made in connection with chromosome-physiological studies in *Colchicum*.

Colchicum has proved to be a rather difficult cytological material. FURLANI (1904) gives the chromosome number of *Colchicum autumnale* as n = 7, and HEIMANN-WINAWER (1919) gives the number n = 10—12 for the same species. Both these numbers are probably erroneous. MILLER (1930) writes: »Although 5 species of *Colchicum* have been examined, no definite number can be given for any of them. The chromosomes would appear to be extremely adhesive, and hang together in chains from 2 to 5 . . . The diploid number so far obtained for *Colchicum* is certainly more than 24, probably more than 32, and quite possibly more than 40 . . . The adhesiveness seen in the chromosomes of the group *Colchicae* is present also in some of the other groups (of the *Melanthioideae* section), although not to such an extent». NEWTON (in TISCHLER, 1931) gives n = 21 for the two species *Colchicum autumnale* and *Parkinsonii*. SUTÔ in his list of Liliaceous chromosomes (1936) mentions *Colchicum* as having the *Lilium-Narcissus* type of its idiogram, i. e. all the chromosomes large and of about the same length. I have not been able to find from where this erroneous description originates, since, as far as I am aware, no pictures or detailed descriptions of *Colchicum* chromosomes have so far been published.

In the present paper 10 *Colchicum* species are examined (Table 1). They were procured from the Botanical Gardens of Lund and Copenhagen by the courtesy of Mr. A. TÖRJE and Dr. O. HAGERUP. All of them belong to the Sectio *Autumnales* (STEFANOFF, 1926), with the exception of *montanum*, the determination of which is not sure. The root tips were fixed in NAVASHIN. In order to diminish the tendency

of adhesiveness of the chromosomes, the fixations were made under cold conditions (at about 0° C), and the plants were kept in a cold greenhouse. The chromosomes were stained in gentian violet. The plants studied showed an abundancy of beautiful mitoses. Nevertheless there was a risk of making erroneous estimations of the chromosome numbers, since the large chromosomes very often had prominent

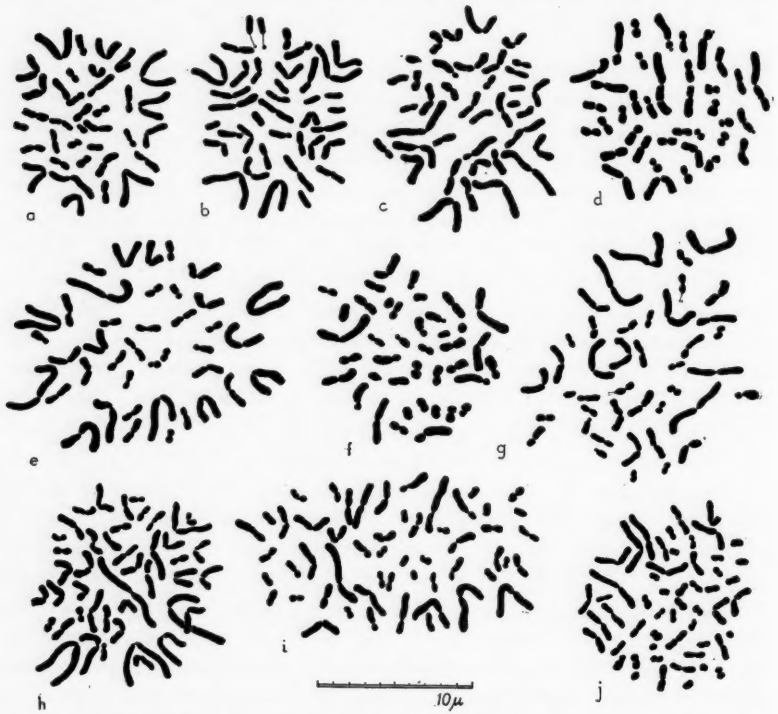


Fig. 1. Somatic metaphase plates of *Colchicum* species, *a*: *Bivonae* (2n = 36), *b*: *autumnale* (2n = 38), *c*: *neapolitanum* (2n = 38), *d*: *speciosum* (2n = 38), *e*: *byzantinum* (2n = 40), *f*: *giganteum* (2n = 40), *g*: *Bornmülleri* (2n = 42), *h*: *variegatum* (2n = 44), *i*: *latifolium* (2n = 54), *j*: *montanum* (2n = 54). — × 2400.

constrictions, due to which a chromosome segment could easily be interpreted as a free, small chromosome. The chromosome numbers given in Table 1 are probably correct, however, although in some forms of *speciosum* 39 were counted instead of 38 chromosomes, and in some plants of *autumnale* the numbers 39 and 40 were counted. Possibly a certain degree of autopolyploidy is present, which, as for instance in

Allium, might cause the origin of clones with a somewhat varying somatic number. The examination of meiosis should settle this question. It is certain, however, that the differences of the idiograms between the species is not only of a numerical nature, but also a structural. In most species, for instance, the largest chromosomes have medially located centromeres, while in some species (e. g. *giganteum*) the largest chromosomes are clearly asymmetric.

The appearance of the chromosomes of the different species is seen from Fig. 1. The variation in size within each idiogram is very prominent. 4—6 chromosomes of each species are very long, up to 7 μ. These chromosomes have ordinarily median centromeric constriction. In certain species 2 of these chromosomes may be recognized by the

TABLE 1. *The Colchicum species examined.*

Species	2n	Pictured in Fig. 1	Material procured from
Bivonae GUSS.	36	1*a*	Copenhagen
autumnale L.	38	1*b*	Lund, Copenhagen
neapolitanum TEN.	38	1*c*	Copenhagen
speciosum STEV.	38	1*d*	Lund, Copenhagen
byzantinum TEN.	40	1*e*	Copenhagen
giganteum hort.	40	1*f*	Copenhagen
Bornmülleri FREYN.	42	1*g*	Lund
variegatum L.	44	1*h*	Copenhagen
latifolium S. S.	54	1*i*	Copenhagen
montanum L.	54	1*j*	Copenhagen

presence of a secondary constriction in about the middle of one arm (Fig. 1 *d*). A great many chromosomes of medium size are present. Their size varies continuously, and I have not made any attempt to classify them. Most of them are more or less medially inserted, but in most species some subterminally inserted chromosomes of the medium size-class occur. In a few cases the shorter arm of these chromosomes is furnished with a satellite (Fig. 1 *d*). Among the small chromosomes there exist differences in size, the smallest are not longer than 0,6—0,8 μ, thus only about one tenth of the longest chromosomes. Also among the small chromosomes the medially attached types predominate, even if purely terminally attached chromosomes seem to occur at least among the smallest chromosomes, which are almost spherical at metaphase. In a couple of species one pair of the small

chromosomes has satellites (Fig. 1 *b*, *g*). The satellites are tiny and the attachment threads are long.

The examples of idiograms of *Colchicum* species given above show that several chromosome numbers occur in the genus. The numbers known so far cannot be arranged in any polyploid series. Neither does the chromosome morphology suggest simple polyploidy conditions. The type of idiogram agrees very closely with the conditions in certain other Liliaceous genera, where long, medium and very small chromosomes are mixed in the same idiogram. The species dealt with in this paper represent only one of the 8 sections of the genus. Their idiograms must be regarded as rather derived. It is possible that the study of the other sections of the genus will reveal species with more primitive idiograms and in this manner furnish a clue as to the evolution of idiograms within the genus *Colchicum*. Such a study is planned, parallel with a study of the reactivity behaviour to colchicine within the whole genus of *Colchicum* and neighbouring genera.

Svalöf, January 13th, 1940.

LITERATURE CITED.

1. Furlani, J. 1904. Zur Embryologie von *Colchicum autumnale* L. — Oesterr. bot. Z. 54: 318—324, 373—379.
2. Heimann-Winawer, P. 1919. Beiträge zur Embryologie von *Colchicum autumnale* L. — Diss. Zürich, 65 p.
3. Levan, A. 1940. The effect of acenaphthene and colchicine on mitosis of *Allium* and *Colchicum*. — Hereditas XXVI: 262—276.
4. Miller, E. W. 1930. A preliminary note on the cytology of the Melanthioideae section of the Liliaceae. — Proc. Univ. Durham Phil. Soc. 8: 267—271.
5. Stefanoff, B. 1926. Monographie der Gattung *Colchicum* L. — Sophia, 100 p.
6. Sutô, T. 1936. List of chromosome number and idiogram types in Liliaceae and Amaryllidaceae. — Jap. J. Gen. 12: 107—112, 157—162, 221—231.
7. Tischler, G. 1931. Pflanzliche Chromosomen-Zahlen. — Tab. Biol. Per. 1: 109—226.

CYTOLOGICAL STUDIES OF DIPLOID AND TRIPLOID POPULUS TREMULA AND OF CROSSES BETWEEN THEM

BY *HELGE JOHNSSON*
INSTITUTE FOR BREEDING FOREST TREES, SVALÖF, SWEDEN

I. INTRODUCTION.

IT was stated by BLACKBURN and HARRISON (1924) that the diploid chromosome number is 38 and the haploid number 19 in *Populus tremula*; these numbers were confirmed by MÜNTZING (1936) and WETTSTEIN (1937). *P. tremula* has the chromosome number 2×19, in common with all other *Populus* species, about fifteen, the chromosome numbers of which are known (BLACKBURN and HARRISON, 1924; MEURMAN, 1925; WETTSTEIN, 1937; PETO, 1938).

The only reported exception has been recorded by BLACKBURN and HARRISON (1924), who consider *P. balsamifera* to be a tetraploid; MEURMAN found (1925), however, the chromosome number $2x = 19$ for this species too.

MÜNTZING (1936) has shown that a clone of *Populus tremula* with gigas properties detected by NILSSON-EHLE (1936) was triploid with $2n = \pm 57$. Two further clones from other localities in Sweden, having morphological features similar to the previously known triploid clone, were shown by TOMETORP (1937) to be approximately triploid. The morphology of one of these clones is described by BLOMQUIST (1937). In Canada, PETO (1938) has reported the occurrence of triploid *P. canescens* and *P. alba*. NILSSON-EHLE (1938) has described the occurrence of a tetraploid seedling in the progeny of a cross $2x \times 3x$ *P. tremula*, and some data concerning these crosses are given by BERGSTRÖM (1940).

In the present paper some results will be discussed, mainly concerning the meiotic behaviour of diploid and triploid *P. tremula* and the fertility and chromosome numbers of the progeny of crosses between diploids and triploids. The work is being carried out from a breeding point of view at the Institute for Breeding Forest Trees at Svalöf, Sweden.

II. MATERIAL AND METHODS.

The original material for the study of meiosis, as well as the crosses made, was collected from wild populations in Sweden. These parental clones are in the main selected by Dr. N. SYLVÉN, Svalöf, and spread all over the country. In the case of triploids their detection is due to reports of extremely large-leafed aspens made by several persons.

In the winter cut twigs were placed in greenhouses, fixations of pmc were made at the proper time, and crosses were performed according to the method described by WETTSTEIN (1937). Pmc:s were prefixed in Carnoy for a short time and then fixed in diluted chrome-acetic-formalin. The sections were cut to a thickness of 14 μ and stained in gentian violet. In some cases the somatic chromosome number, too, was determined for the parent clones, and then in root tips from suckers, planted in pots, or — in the case of some females — in the carpels.

On determining the 2n-number of seedlings the plants were planted in pots, and root tips fixed in diluted chrome-acetic-formalin. Sections, 10 μ thick, were cut and stained in gentian violet. In this connection some observations made as to the most suitable time for fixation may be mentioned briefly. Three series of fixations of the same plant material were made on different days under different weather conditions. The fixations were performed every other hour of the 24 hours. It appeared that fixations made at noon in sunlight gave numerous divisions, but the divisions were unclear without distinct metaphase plates. On the other hand, fixations made in the early morning or on cold, rainy days yielded few divisions but with pronounced metaphase plates, more suitable for counting. This result may be said to agree with the observations of KIHARA that an artificial cooling of the plant before fixation gives shorter chromosomes, more evenly distributed in the plate. On account of this superior result of fixation early in the morning the whole of the present material was fixed at that time.

In measuring the diameters of pollen grains a 40 objective and a 7x eyepiece were used, one unit = 3,8 μ. In measuring the stomata lengths the same system was partly used, partly a 40 objective and a 15x eyepiece, one unit = 2,4 μ. The drawings were made with a camera lucida, using a 100 fluorite objective and a compensating 30x eyepiece, giving a magnification of $\times$ 6000, which is reduced to $\times$ 4100. The microphotographs were taken with a Zeiss »Standard» camera.

III. TRIPLOID ASPEN.

1. OCCURRENCE.

At present 9 different clones of triploid aspen are known in Sweden. These clones are growing in the following different localities.

1. Lillö, Skåne, South Sweden (NILSSON-EHLE, 1936; MÜNTZING, 1936).
2. Våle, Medelpad, Middle Sweden (BLOMQUIST, 1937; TOMETORP, 1937).
3. Vittjärv, Norrbotten, North Sweden (TOMETORP, 1937).
4. Våle, Medelpad, Middle Sweden, in the neighbourhood of 2.
5. Vittjärv, Norrbotten, North Sweden, in the neighbourhood of 3.
6. Jansjö, Ångermanland, Middle Sweden.
7. Häggnäs, Västerbotten, North Sweden.
8. Rosinedal, Västerbotten, North Sweden.
9. Vänsjärv, Norrbotten, North Sweden.

In most cases the 2n- as well as n-numbers have been determined, and the triploid behaviour is recorded by crossing with diploid partners. In no cases, however, have the chromosome numbers been ascertained to be exactly 57, but may be assumed to be $\pm$ 57; a difference of one chromosome may be possible but rather unlikely. In Fig. 18 a somatic plate with 57 chromosomes is drawn.

2. CELL SIZE.

The criteria adopted when searching for triploid aspens were extremely large leaves. MÜNTZING (1936), TOMETORP (1937) and BERGSTRÖM (1940) state that triploid aspens have longer stomata than

TABLE 1. *Size of the leaves, lengths of stomata and chromosome number in wild P. tremula.*

Leaves	2n	Lengths of the stomata										Mean	No. of trees
		6,6—7,0	7,1—7,5	7,6—8,0	8,1—8,5	8,6—9,0	9,1—9,5	9,6—10,0	10,1—10,5	10,6—11,0	11,1—11,5		
Small	38		2	4	1							$7{,}7 \pm 0{,}13$	7
»	—	5	38	63	19							$7{,}7 \pm 0{,}03$	125
Large	38				1	1					1	$9{,}5 \pm 0{,}93$	3
»	57				4	9	1	1				$8{,}7 \pm 0{,}10$	15
»	—			4	3	7	1					$8{,}5 \pm 0{,}13$	15

diploid ones. In Table 1 the correlation between size of the leaves, length of stomata and chromosome number are shown for the present

material, which also includes the triploids of MÜNTZING and TOMETORP. In estimating the length of stomata, 50 stomata from each of three leaves from every tree were measured. One of the three leaves was extremely large, one medium and one small, but all belonged to the larger leaves of the tree. The length of the stomata vary much within the leaf; consequently the calculated mean, tabulated as characteristic of the tree, cannot be of greater accuracy. 1 unit is equal to 3,8 μ. It will be seen that all small-leafed trees have short stomata and as far as known $2n = 38$. The large-leafed trees have on an average larger stomata than the small-leafed ones, but as regards the chromosome number the large-leafed trees with long stomata are diploids as well as triploids. Taking the length of stomata as a measure of cell size one must conclude that *the size of the leaves depends on the size of the cells. The size of the cells in turn depends on chromosome number* (triploids having larger cells than most diploids) *and on other presumably hereditary factors not known* (there are diploids having as large cells as the triploids and even larger). That there is a correlation between size of the leaves and stomata length (cell size) in the individual tree, too, is illustrated by the means of the stomata length of the respective large, medium and small leaves of all the 165 trees measured.

Size of the leaves:	large	medium	small
Mean length of the stomata:	7,90	7,88	7,79 units

Thus the easily recognizable variation in leaf-size within the tree is associated with a variation in cell size, and in consequence *the cell size is influenced by modifying factors, too. Thus the length of the stomata as a measure of cell size in aspen is of no higher value as an indicator of triploidy than the leaf-size per se.* However, the leaf-size is of value in searching for triploid aspens as a means of eliminating non-triploid trees. For — in addition to the small number of small-leafed trees, which have been 2n-determined — a great number of such trees has been used in crossing, and all of them have given perfectly uniform progenies; not a single one has given the variable offspring, characteristic of triploid parents. Thus it seems rather unlikely that small-leafed triploid aspens exist.

3. POLLEN PROPERTIES.

MÜNTZING (1936) and TOMETORP (1937) have shown that triploid aspens have larger pollen grains and poorer pollen than diploid ones. Working with triploid *P. alba* and *P. canescens* and with diploid species and hybrids with meiotic disturbances, PETO (1938), however, arrived

at the conclusion that in his material no relation exists between either chromosome numbers or meiotic behaviour and size of the pollen grains and quality of the pollen.

Data relating to the pollen size and quality of the present material are given in Tables 2 and 3. Table 2 shows the diameter of the pollen grains. Every measurement comprises 100 pollen grains, 1 unit = 3,8 μ

TABLE 2. *Size of the leaves, chromosome number and diameter of the pollen grains in wild P. tremula.*

Leaves	2n	Diameters of the pollen grains						Mean	No. of trees
		9,1— 9,5	9,6— 10,0	10,1— 10,5	10,6— 11,0	11,1— 11,5	11,6— 12,0		
Small ...	38		4	3	6			10,5 ± 0,11	13
» ...	—	3	18	14	4	1		9,9 ± 0,07	40
Large ...	38				1			10,8	1
» ...	57			2	5		1	10,8 ± 0,13	8
» ...	—	1	3	2	2			9,8 ± 0,18	8

The small-leafed trees with unknown 2n-numbers may for very good reasons be considered as diploids. Then it is obvious that the triploid aspen has, on an average, larger pollen grains than the diploid one.

TABLE 3. *Size of the leaves, chromosome number and per cent good pollen in wild P. tremula.*

Leaves	2n	Percentage of good pollen													Mean
		30	35	40	45	50	55	60	65	70	75	80	85	90	95	
Small ...	38			1		1	1	1	3	5	1			2	5	74,0 ± 4,2
» ...	—						1		2	3	6	5	5	6	8	76,5 ± 1,7
Large ...	38											1			1	87,5
» ...	57	1			1	1	1			2	2					58,8 ± 4,7
» ...	—						1				1	3	1			75,8 ± 4,4

The two populations, however, overlap each other. Consequently there is no possibility of ascertaining whether the large-leafed trees with unknown 2n-numbers are diploid or triploid with the aid of the pollen diameter. MÜNTZING (1936) presents a bimodal curve for the pollen diameter of triploid aspens. In the present material no trace of such bimodality has been observed, but occasionally bimodality may occur (see below and Fig. 13). Table 3 gives the quality of the pollen. Every estimate is as a rule based on about 200 pollen grains. However, there

is no sharp boundary between »bad» and »good» pollen. — MÜNTZING works, obviously for that reason, with three categories: »good», »dubious» and »bad» grains. — Owing to this fact the estimates must be considered to be rather uncertain. Furthermore, the sampling error of each estimate must be high. There is a wide range of variation in percentage of good pollen among the trees of every category. Of course this variation is partly due to errors, but undoubtedly there is a significant variation, too. This variation may be due to external or internal factors. On an average, however, the triploids have poorer pollen than the diploids. But as in the case of the pollen grain diameter the variations overlap.

The inference must be that *the triploids have large pollen grains and poor pollen*, but many diploids have as large pollen grains and as poor pollen as many triploids — at least when the estimates are based on only one or a few samples.

However, the distinction between diploid and triploid pollen is somewhat more sharply defined, when both diameter and quality are considered together. Further, in the triploid pollen there is sometimes one or other elongated, sometimes constricted grain. Thus the pollen sample has a limited value in determining whether an aspen is diploid or triploid.

4. SEX.

Of the reported nine triploid aspens four are pure males, three pure females and two the sex of which is unknown. Nothing is known about the sex determining mechanism of the diploid aspen. But assuming this to be the type of a monofactorial back-cross, we may for convenience denote the one sex with XY and the opposite sex with XX. The triploid aspen must have originated as a result of a fusion between one unreduced and one reduced gamete, as MÜNTZING (1936) points out. Then if the unreduced gamete is of the XY-sex, the result must be one XXY-triploid. If, on the other hand, the unreduced gamete is of the XX-sex triploids of two constitutions may be produced, viz. XXX and XXY. Thus we have two different constitutions of the triploids as far as sex determinators are concerned. If the frequency of unreduced gametes is the same in both sexes of the diploid, and the chance of surviving is the same for both male and female unreduced gametes, the numerical relation between the two triploid types should be 3 XXY : 1 XXX. The XXX-type must be expected to be of one pure sex. The XXY-type may be of the same or the opposite sex or an intersex. Now we have pure males and pure females among the triploids, but no

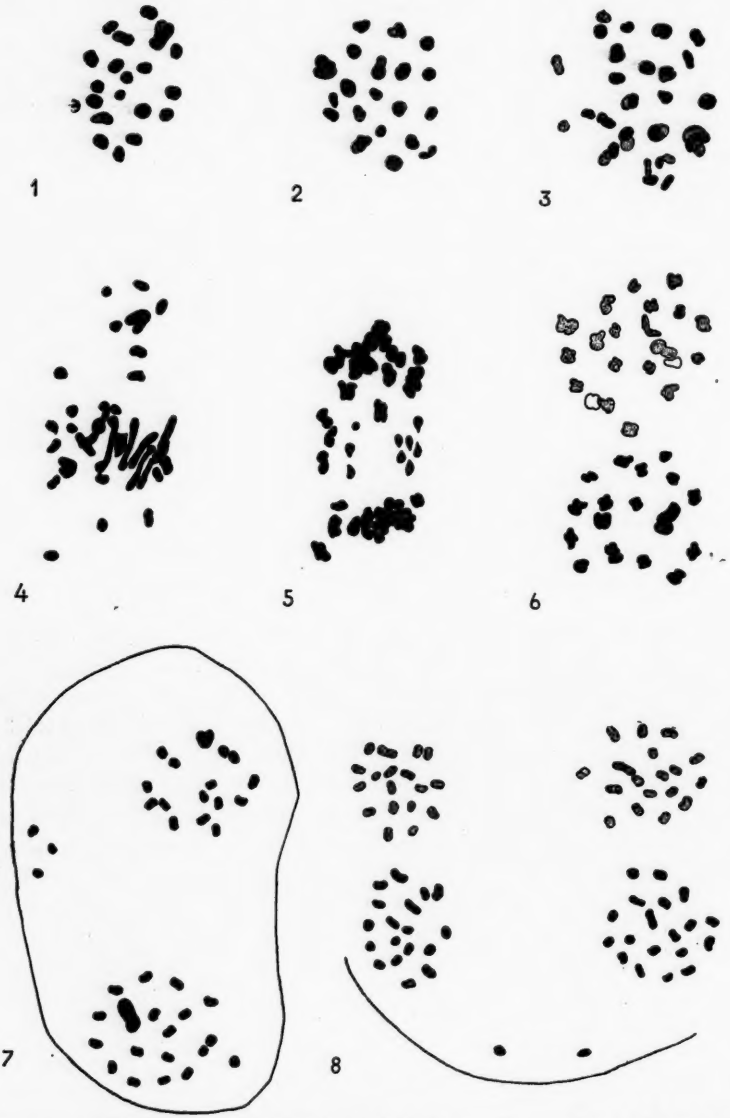
intersexes. Thus one of the triploid sexes may be assumed to have the constitution XXX and the other XXY. This is in agreement with WARMKE and BLAKESLEE's (1939) result on triploid *Melandrium*, i. e. 3A + XXX individuals are females and 3A + XXY individuals are males. However, one 3A + XXY-plant was actually an intersex.

IV. MEIOSIS OF DIPLOID ASPEN.

In their study of the meiosis of diploid aspen, BLACKBURN and HARRISON (1924) point out that the chromosomes were unequal in size: »Nine small ones of more or less uniform size; nine others, larger than them, formed a graded series beginning with a member of just a little greater volume than the individual of the first group and ending with one more than four times its volume. Lastly there was a single chromosome . . . equalling in volume if not exceeding that of any two of the other eighteen». This description is, indeed, very striking, especially the occurrence of one bivalent much bigger than the others (cf. MÜNTZING, 1936). In Fig. 1 a IM in polar view is drawn, showing this quite clearly. It is impossible to determine the bivalent condition of all bodies in polar view. In Fig. 9 a photograph of a IM is shown. Later on meiosis behaves quite schematically. BLACKBURN and HARRISON (1924) assume that there is a pair of heterochromosomes present in *P. tremula.* At any rate the difference cannot be great between the members of this pair, and as far as my experiences go, it will be exceedingly difficult to ascertain the presence of such a pair with but slightly differentiated members in *P. tremula.* The bivalents are small and numerous, in side view for the most part intensely crowded. The occurrence of occasional bivalents, asymmetrical in shape, may be due to chance.

For some other *Populus* species heterochromosomes have been reported by MEURMAN (1925) and BLACKBURN (1929). PETO (1938), treating *P. tremuloides,* heterochromosomes of which are reported (ERLANSON and HERMANN, according to PETO), states, however, that »no definitely heteromorphic pair has been found consistently».

Out of 16 clones, the meiosis of which has been studied, 12 behaved quite normally, but the remaining four showed a varying number of chromosome bodies in polar view of IM, ranging from 19 to as many as 31. Thus in Fig. 2 there are 20 quite clear »separate bodies», while in Fig. 3 there are 26. No doubt, these clones, too, have the exact diploid chromosome number. For at all events some IA and IIM plates



Figs. 1—8. Meiosis with univalents in diploid *P. tremula*. — Fig. 1, IM with 19 bivalents; Fig. 2, IM with 20 separate bodies, bivalents and univalents; Fig. 3, IM with 26 separate bodies, bivalents and univalents; Fig. 4, IM in side view, with 8 bivalents and 22 univalents; Fig. 5, IA with lagging univalents in division; Fig. 6, IA in polar view (the two plates separately drawn) with 18—19—2 chromosomes; Fig. 7, IIM with 19—16—3 chromosomes; Fig. 8, IIA with 19—18 : 19—18 : 2 chromosomes (the sister plates separately drawn).

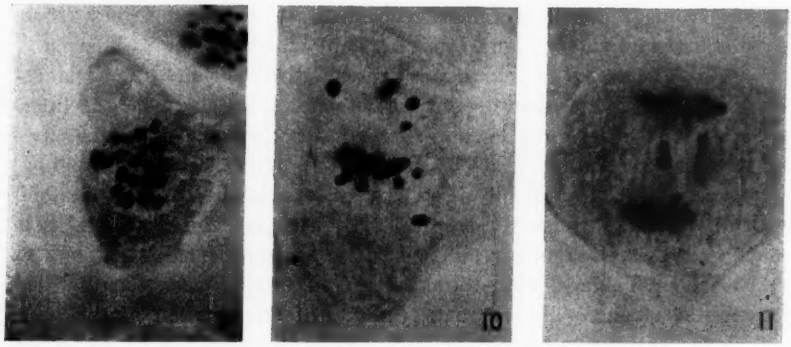
have shown the sum of 38 chromosomes. In side view of IM, Fig. 4 and photograph, Fig. 10, it is quite clear that only bivalents and univalents occur (two of the univalents are evidently the members of the large chromosome pair). Thus Fig. 2 should represent $18_{II} + 2_{I}$ and

TABLE 4. *The frequency of univalents in some diploid aspens.*

Origin of the clones	Number of separate bodies per pmc													Mean	% good pollen
	19	20	21	22	23	24	25	26	27	28	29	30	31		
Östergötland	1	5	5	2	0	1								20,9	70,0
Uppland	6	2	4	1	0	0	1							20,4	38,9
Medelpad.....	4	2	1	0	1									20,0	80,3
Hälsingland	1	2	3	3	1	0	2	2	1	0	0	0	1	23,2	67,1

Fig. 3 $12_{II} + 14_{I}$. In Table 4 some figures are given, showing the degree of asyndesis of the four clones. It will be seen that the frequency of univalents is especially high in the clone from Hälsingland.

At IM the univalents are distributed irregularly in the cell (Fig. 4



Figs. 9—11. Meiosis in diploid *P. tremula*. — Fig. 9, IM in polar view with 19 bivalents; Fig. 10, IM with univalents in side view; Fig. 11, IA in side view with lagging univalents.

and photograph, Fig. 10). Frequently some are situated in the polar regions. At IA a larger or smaller number of bodies, certainly univalents, lag behind and divide or begin to divide (Fig. 5 and photograph, Fig. 11). Fig. 6 shows a IA in polar view (the plates are drawn separately). In one plate there are 18 chromosomes, in the other 19 and another 2 are situated at one of the poles. This IA must have been

brought about by division of one univalent and may be interpreted as the result of a IM with $17_{II} + 4_{I}$, one univalent of which has divided, and the halves passed to opposite nuclei, one univalent has been included undivided in the one nucleus, and the other two univalents have remained undivided in the polar region outside the plate. These two univalents may or may not be included in a nucleus at interphase. In most cases the lagging bodies do not arrive at the poles before the first division is finished, and the cells enter the resting stage. Evidence of such an elimination is shown in Fig. 7 (a IIM) where two plates with 19 and 16 chromosomes respectively are seen, and three more chromosomes are situated between the nuclei and close to the inside of the cell wall. Probably these chromosomes are undivided univalents, for the sum of the chromosomes is exactly 38. In Fig. 8 two chromosomes opposite to each other near the wall are situated apart from the plates. A univalent may have passed through the first division undivided, not being included in any of the nuclei and then passes through the second division separately. In this figure (the sister plates are drawn separately) one of the two pairs of plates at IIA has 19 and the other 18 chromosomes. This fact may be understood if each of the daughter plates from first division has received 18 split chromosomes (from bivalents or univalents) and one univalent which has split later on.

Thus, in *Populus tremula* univalents divide or do not divide at first division. The split or unsplit univalents are included in interphase nuclei or are left outside them. At second division split chromosomes, included in a nucleus, pass to one of the two nuclei without further division. The split chromosomes, excluded from the daughter nuclei at IA can finish the second division separately. Indications of asynaptic behaviour in diploid *P. tremula* were reported by MÜNTZING (1936). In Table 4 estimations of pollen quality are given for the asynaptic aspens. The mean percentage of good pollen of the other 12 clones with quite regular meiosis was 79,9. Thus it is seen that the variation in pollen quality in diploid aspen can to some degree be attributed to meiotic disturbances.

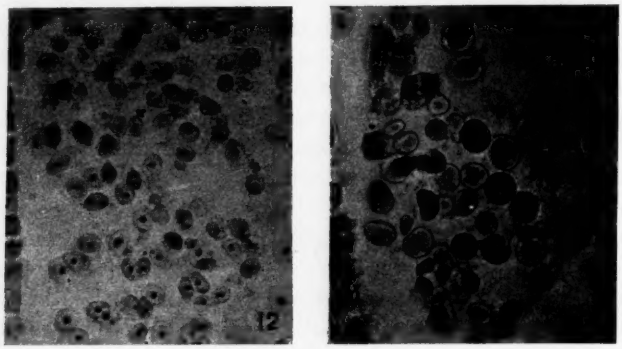
With regard to the occurrence of univalents in diploid aspen several causes may be taken in consideration. Structural hybridity seems very unlikely. In Sweden no other wild *Populus* species than *P. tremula* occurs. That structural changes within the species should take place, involving so many chromosome pairs and without multiple associations, seems hardly plausible. Genes causing an omission of chiasma formation are known in several plants (maize, BEADLE and MC CLINTOCK,

1928; *Datura*, BERGNER et alii, 1934; *Alopecurus*, the present writer, in press). The most probable cause, however, is to be sought in external influences. STRAUB (1937) has shown that changes in temperature at leptotene and pachytene in *Gasteria trigona* affect the chiasma formation and result in a varying number of univalents at IM. Pmc:s of examined aspens were fixed from twigs, which only a few days previously (in January and February) had been cut out-of-doors and transferred to a greenhouse. Probably pmc:s of some clones were at that very moment in a sensitive stage. Outdoor pmc:s pass through meiosis in early spring at low temperature. No doubt considerable changes in temperature may sometimes occur in nature at that time. Thus if the occurrence of univalents in aspen is connected with changes in temperature univalents will also arise in nature. On the other hand, an occurrence of univalents in high frequency will furnish special opportunities for the formation of unreduced gametes. Owing to the fact that triploid aspen is not especially rare, unreduced gametes must be regarded as fairly common in nature.

V. MEIOSIS OF TRIPLOID ASPEN.

A description of the course of meiosis in the triploid male aspen from Skåne has been given by MÜNTZING (1936). This author has established that IM of the triploids is characterized by a varying number of trivalents and univalents in addition to bivalents, and that chromosomes are often lagging at IA and may then constitute a connection between the nuclei, which may give rise to unreduced gametes. Further, he has proved that irregularities may occur even at the second division. — The present writer has studied meiosis of the same triploid as MÜNTZING, and in addition the meiosis of two triploid male clones growing in Medelpad in the neighbourhood of each other and also the meiosis of a few available emc:s of the triploid female clone from Norrbotten. In all cases examined meiosis was of the same appearance as that described by MÜNTZING. On account of the small and rather numerous chromosomes it is impossible to give any detailed statistics with respect to the frequency of trivalents, the amount of chromosome elimination, chromosome numbers of the first and second anaphase and so on. On the whole, the formation of interphase nuclei and tetrads seems to proceed rather regularly and the resulting young pollen grains appear uniform in size, having one nucleus (photograph, Fig. 12). However, most of the pollen grains contain chromatin bodies outside

the nucleus. These bodies are probably derived from lagging and eliminated chromosomes, which almost invariably can be seen in the first as well as in the second anaphase. The pollen nuclei must be presumed to have varying chromosome numbers. Infrequently a loculus in one or other stamen of a flower presents a quite different picture, as illustrated by the photograph, Fig. 13. In these cases the pollen grains are of very different size, some being normal-sized, some much larger. The large pollen grains may have one, two, three or four nuclei. These facts make it clear that meiosis and the formation of cell walls are influenced by external or internal factors, affecting



Figs. 12—13. Young pollen grains of triploid *P. tremula*. — Fig. 12, one loculus with uniform grains of normal size; Fig. 13, one loculus with unequal-sized grains, mainly large grains with one, two or three nuclei.

only parts of a flower. No doubt, these large pollen grains with one or more nuclei have higher chromosome numbers than is usually the case, and if they are capable of functioning they will give rise to offspring with more than the triploid chromosome number.

VI. CROSSES BETWEEN DIPLOID AND TRIPLOID ASPEN.

In the winter of 1938 a number of crosses between diploid and triploid aspen were made according to the method described by WETTSTEIN (1937). As triploid parents the male triploid from Skåne and Medelpad, used by BERGSTRÖM (1940), and the female triploid from Norrbotten were employed. As diploid parents clones from different localities in Sweden were selected. A total of 29 crosses yielded a varying number of offspring plants.

The seedlings of these crosses show a very characteristic behaviour,

TABLE 5. *Chromosome numbers of the progeny of crosses between diploid and triploid P. tremula.*

Triploid parent	Cross number	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	± 4x	Mean 2x—3x	% 2x	n
♂ Skåne ...	62	23	5	2	3	4	1	2	5	6	2	7	7	4	8	3	7	4	4	0	1	1	45,9 ± 0,67	23,5	99
» ...	200	2	0	0	0	0	0	0	1	0	0	1	0	2	0	0	0	0	0	1	0	13	46,4 ± 2,51	28,6	20
» ...	203	11	5	1	3	0	1	0	1	0	0	2	1	0	0	0	0	0	0	1	0	4	41,0 ± 0,81	42,3	30
» ...	215	9	0	1	3	0	1	2	0	1	3	2	1	4	0	0	1	1	3	2	0	0	45,7 ± 1,08	26,5	34
» ...	254	15	4	2	4	8	9	8	4	9	17	19	10	16	26	8	8	4	3	1	1	0	47,2 ± 0,35	8,5	176
» ...	293	6	1	0	1	2	2	2	1	2	1	8	3	7	5	5	9	1	4	0	2	0	48,5 ± 0,20	9,7	62
» ...	S 10 numbers	13	2	6	2	3	0	0	2	5	3	7	6	3	2	0	1	2	2	2	1	0	45,0 ± 0,73	21,0	62
Total		79	17	12	16	17	14	14	14	23	26	46	28	36	41	16	26	12	16	7	5	18	46,3 ± 0,26	17,0	483
♂ Medelpad	297	7	0	3	1	0	3	1	1	0	1	1	2	1	1	7	1	1	0	0	3	0	46,5 ± 1,12	20,6	34
»	304	1	0	1	0	0	0	0	0	0	0	0	0	1	0	2	0	0	1	0	0	0	47,8 ± 2,88	16,7	6
»	311	3	1	0	3	0	1	0	2	0	1	1	2	1	0	2	0	1	0	1	0	0	45,6 ± 1,33	15,8	19
Total		11	1	4	4	0	4	1	3	0	2	2	4	3	1	11	1	2	1	1	3	0	46,3 ± 0,79	18,6	59
♀ Norrb....	184	2	0	3	0	0	1	0	2	3	3	2	2	2	0	2	0	0	0	0	0	0	45,6 ± 0,92	9,1	22
»	223	2	1	1	0	3	1	1	0	1	4	1	2	3	1	1	2	2	0	0	0	0	46,7 ± 0,98	7,7	26
»	230	2	0	0	0	0	0	0	2	0	1	0	0	0	2	0	0	1	1	0	0	0	47,1 ± 1,97	22,2	9
»	S 7 numbers	3	1	1	0	1	0	1	0	1	1	5	0	2	0	0	0	1	1	0	0	0	45,6 ± 1,27	16,7	18
Total		9	2	5	0	4	2	2	4	5	9	8	4	7	3	3	2	4	2	0	0	0	46,2 ± 0,57	12,0	75
Total		99	20	21	20	21	20	17	21	28	37	56	36	46	45	30	29	18	19	8	8	18	46,3 ± 0,23	16,5	617
Calculated Total, surviving plants, Aug. 1939...		0	0	0	1	4	13	31	58	86	106	106	86	58	31	13	4	1	0	0	0		47,5	0,0	599
		88	18	15	15	19	16	14	15	20	27	47	25	39	43	23	25	15	15	7	7	15	46,2 ± 0,20	17,8	508
% loss		11,1	9,0	28,6	25,0	9,5	20,0	17,7	28,6	28,6	27,0	16,1	30,6	15,2	4,4	23,3	13,8	16,7	21,1	12,5	12,5	11,8 ± 2,2			
		0,3	19,0 ± 0,1																						

compared with seedlings from crosses between diploid parents; the latter are uniform in size and appearance (Fig. 14), the former varying in size, in the shape and thickness of their leaves and so on (Fig. 15).

Figs. 14—15. Seedlings of *P. tremula*. — Fig. 14, Progeny of a cross 2x — 2x aspen; Fig. 15, Progeny of a cross 2x — 3x aspen.

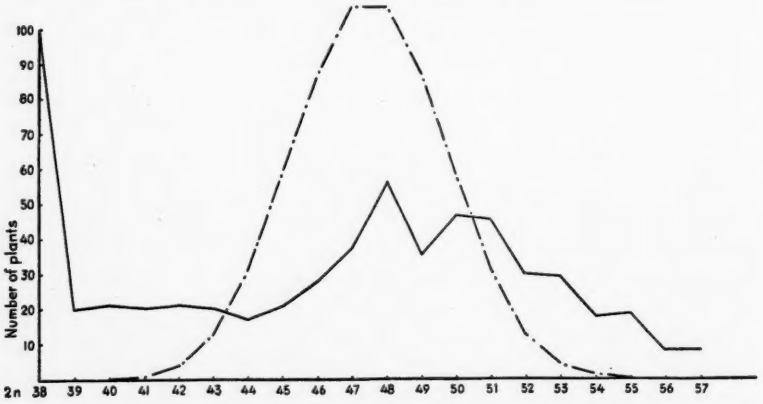
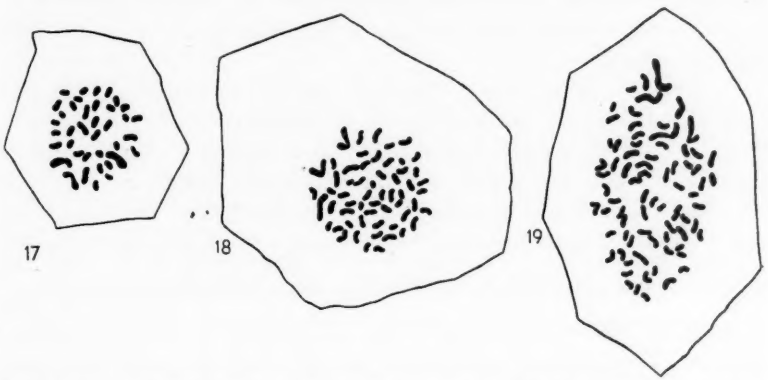


Fig. 16. Diagram showing the experimental distribution of the 2n-numbers in crosses between diploid and triploid *P. tremula* (———) compared with the binomial distribution (—·—·—).

The chromosome numbers have been determined without selection for as many plants as possible in the offspring, and the result is shown in Table 5. These chromosome numbers, however, are not determined with absolute accuracy; the error is on an average ± 1 chromosome. The reason for this is the great difficulty of making an accurate count of the somatic chromosome numbers (cf. MÜNTZING, 1936 and BERGSTRÖM, 1940) owing to the fact that practically in all plates the very small chromosomes, at least at some point, are crowded, making a clear distinction impossible, or the median constriction of some chromosome may be long and uncoloured, so that this chromosome might have been counted as two. In the determination of a chromosome number



Figs. 17—19. Somatic plates of *P. tremula*. — Fig. 17, $2n = 38$; Fig. 18, $2n = 57$; Fig. 19, $2n = 76$.

the three best plates were counted, and when the results have differed by one, two or occasionally more chromosomes the mean was taken. In Figs. 17—19 some somatic plates are drawn.

One of the most striking features is that all chromosome numbers between 38 and 57 are represented (cf. BERGSTRÖM, 1940). In general, in crosses between diploids and triploids the intermediate chromosome numbers are very rare, if represented at all, in the progeny (DARLINGTON, 1937; UPCOTT and PHILP, 1939). On the other hand, almost all offspring plants have the diploid or a slightly higher chromosome number. That is true, even when the data of a large progeny are given (BELLING and BLAKESLEE, 1922, on *Datura*). But recently UPCOTT and PHILP (1939) were able to show that the progeny from 3x — 2x crosses in *Tulipa* had the intermediate 2n-numbers very well represented, and

the authors state: »These data provide the only example of triploid-diploid progeny with chromosome numbers evenly distributed between those of their parents». However, similar results have been previously reported, viz. between diploid and triploid apple. The data were recently summarized by WANSCHER (1939). This case is according to DARLINGTON (1937) a very special one and is considered by him to accord with the view he arrived at in other ways, that the x-number 17 in *Pyrus* is a secondary one.

The mean 2n-number for the whole material of 599 plants, ranging between 38 and 57, is $46{,}3 \pm 0{,}23$. Different crosses have, of course, a somewhat different mean, but in view of the standard errors the differences are not significant, perhaps with the single exception of cross No. 203, which seems to have a significantly lower mean value than the others.

MATHER (1935) states that the odd chromosomes in triploid *Triticum* at meiosis are distributed at random and in consequence gametes must be produced with binomial frequencies with n-numbers ranging between x and 2x. UPCOTT and PHILP (1939) have worked with the chromosome numbers of the pollen grains in *Tulipa* and summarized the literature, and they show that this law seems to have a general application. The mean n-number of the pollen-grains is $\frac{3x}{2}$ or slightly below, according to the elimination of chromosomes at meiosis. There are no reasons for believing that 3x-aspen behaves in a different manner. Consequently the progeny of crosses diploid—triploid aspen should have $\frac{2x + 3x}{2}$, i. e. $47{,}5$ chromosomes on an average; in fact, the values is $46{,}3 \pm 0{,}23$ or somewhat lower than expected. This slight difference may be inferred as being due to chromosome elimination at meiosis or to the limited accuracy with which the chromosome numbers are determined, or to both facts in co-operation. However, another effect of a random distribution of the univalents at meiosis is that the 2n-numbers of the progeny must also be distributed in binomial frequencies. The binomial frequencies of 599 variants are given in Table 5. No doubt, the calculated and the experimental values do not agree at all. This fact is further illustrated in the diagram, Fig. 16. In spite of the fact that all intermediate 2n-numbers are realized and that the mean 2n-number is only slightly below the calculated, the distribution differs very much. The intermediate 2n-numbers, from 44 to 50, are deficient and the extremes,

38—43 and 51—57, are in excess. Especially the excess of 2n = 38 is very great, 99 plants or 16 % having this 2n-number. According to the law of chance only 1 plant in 524,288 should have had this 2n-number, i. e. very likely none of the present 599 plants. The percentage of 2n = 38 plants varies between different crosses within rather wide limits, being only 7,7 % for Cross 223 and 42,3 % for No. 203.

The general explanation of the discrepances between calculated and experimental values in crosses of this type is that elimination of gametes or zygotes or both takes place (cf. DARLINGTON, 1937). Probably this is true of the aspen crosses too. There are even some data indicating the course of this elimination. At meiosis a certain amount of chromosome elimination occurs; this may imply a tendency to decrease the 2n-numbers of the offspring. As shown in Table 3, the triploids have on an average only 58,8 ± 4,7 % morphologically good pollen grains, varying between 30 and 75 % for different clones and even varying as much as 50 % within the same tree; this variation may be due partly to sampling errors, partly to real differences. Even within the diploid aspen the percentage of good pollen grains varies, but for the best trees it is almost 100 %, and on an average higher than for the triploid.

This must indicate a certain amount of gamete elimination occurring in the triploid male aspen. This elimination may be calculated at about 40 % of the pollen grains.

TABLE 6. *Number of seeds per capsule and germination percentage in crosses 2x × 2x and 2x × 3x.*

2x — ♀ No.	Seeds per capsule		Germination %		Number of crosses	
	× 2x	× 3x	× 2x	× 3x	× 2x	× 3x
3	12,0	6,5	100	62	3	1
26	10,1	5,3	94	90	3	1
47	7,3	3,7	98	98	2	1
54	8,5	5,4	95	98	6	3
100	9,5	6,1	100	90	3	1
101	12,2	3,7	78	91	1	1
Mean and total...	9,9	5,1	94	88	18	8

In Table 6 the seed-setting per capsule and the germination percentage of the seeds are given for crosses between the same mother trees and diploid and triploid males respectively. In estimating the

number of seeds per capsule the seeds of 10 mature capsules were counted under the stereomicroscope with a magnification of 10 times, and the mean was calculated. The tabulated values are then the mean of all crosses with this mother tree; the number of crosses are given on the left side of the table. All six mother trees produced considerably fewer seeds in crosses with triploid males than with diploid ones. The average values are 9,9 seeds for 2x—2x crosses and 5,1 seeds for 2x—3x crosses, that is, an elimination of 52 %. Moreover, the seeds of the 2x—3x crosses did not in general germinate as well as the seeds of the 2x—2x crosses, the mean being 88 and 94 % respectively. The difference is, however, not significant. The main cause of this lack of significance is probably the great sampling error, for the germination tests were made with 1×100 seeds only. — At any rate it may be stated that there is an elimination of at least 50 %. This elimination is probably entirely or to the greatest extent an elimination of zygotes and embryos. That some ovules were not fertilized owing to insufficient pollen tube growth seems to be unlikely; the pollination was performed with a brush and every stigma was powdered with a large amount of pollen grains. — This fertility, reduced to about 50 %, must at any rate be regarded as surprisingly high. UPCOTT and PHILP (1939; cf. DARLINGTON, 1937) state that it is possible »to predict the fertility of a given 2x—3x cross from the 2n-numbers of the parents». They argue that the majority of 2x—3x offsprings are 2x-plants, and that the proportion of x-pollen grains depends upon x. In the case of aspen x is = 19, and only 1 pollen grain in 524,288 is haploid. Consequently, the fertility ought to be very low, but on the other hand the fertility is rather high, the reason being that the aspen is an exception to the law »that the majority of 2x—3x offspring is 2x-plants». The variation in percentage of 2x-plants in different crosses may consequently be inferred as being due to different viability of descendants with intermediate 2n-numbers. Then a high percentage of 2x-plants should be connected with low fertility. Indeed, this percentage is high just in those crosses where the number of counted plants is small. The number of 2n-determined plants, in turn, will be rather strongly correlated to the largeness of the progeny, i. e. to the fertility of the cross.

In Table 7 seed-setting and quality for reciprocal crosses 3x—2x are given. The average seed-setting is only 3,9 seeds per capsule, compared with 5,1 for the 2x—3x crosses; further, the germination percentage is reduced to 58. It is not likely that the 3x females have fewer ovules than the 2x ones, rather the contrary, for the triploid capsules

are larger than the diploid ones. Then the reduced fertility has to be attributed to elimination. This elimination must be the sum of gamete- and zygote-embryo-elimination. Assuming that the gamete- and zygote-elimination is of the same magnitude in 3x—2x crosses as in 2x—3x crosses it is possible to calculate an expected fertility for the 3x—2x crosses, based on the values of the 2x—3x crosses. Starting with 9,9 as the potential number of ovules, there is at first 40 % gamete elimination. 5,94 ovules remain to be fertilized, 47,5 % of these are eliminated as zygotes or young embryos. The germination percentage was 88 %, i. e. a further elimination of 12 % takes place as an elimination of old embryos, and thus the final value will be 2,75 young seedlings per capsule. The experimental value was 3,9 seeds per capsule, 58 % of which germinated, i. e. 2,26 seedlings per capsule. Thus

TABLE 7. *Number of seeds per capsule and germination percentage in crosses 3x × 2x.*

3x — ♀ No.	Seeds per capsule × 2x	Germination % × 2x	Number of crosses × 2x
67	3,3	65	1
56	4,4	50	7
Mean and total	3,9	58	8

the experiment gave a somewhat lower value than that calculated, but this may easily be due to the rather high error occurring in the estimation of good pollen (58,8 ± 4,7 %) and, of course, all the other values, too, have more or less sampling errors. Thus it seems that *the gametic abortion is of about the same magnitude in both sexes of the triploid aspen and that the elimination of zygotes and embryos is of about the same magnitude in both the reciprocal crosses between diploid and triploid aspen.* This result agrees with the *Pyrus* crosses (NEBEL, 1933; cf. WANSCHER, 1939), but is contrary to the bulk of diploid, triploid crosses (cf. DARLINGTON, 1937), where the 3x—2x direction yields a somewhat wider range of variation in 2n-number of the progeny than the 2x—3x direction does.

However, there is a striking difference between the germination percentage of reciprocal crosses. When the triploid is the male parent, the seeds germinate to 88 %, but when the triploid is the female part the germination percentage is only 58 %. This may indicate that the

elimination of embryos takes place later in 3x—2x crosses, the ovules having had time enough to develop so far as to be counted as seeds before the embryos die.

A further plant elimination occurred before the fixations were made. The seeds were sown in boxes, after about four weeks all seedlings or some of them were transplanted into other boxes — without selection — in order to give more space to the plants. After another four-week-period the plants were transplanted again, now to pots in order to produce root tips for fixation, and as many of these plants as yielded roots were fixed. In all these instances plant elimination occurred. In the boxes a large amount of the seedlings died, mainly because of attacks by fungi, and later some plants died with or without

TABLE 8. *Plant elimination in offspring of crosses* $2x \times 3x$.

Plant categories	Number	%
Planted in pots, spring 1938	2369	100
Fixed, summer 1938	962	40,6
Counted	617	26,0
Counted surviving, autumn 1939	508	21,4
Non-fixed + fixed but non-counted, surviving, autumn 1939	669	28,2
Total surviving, autumn 1939	1177	49,7

external symptoms. In Table 8 a survey is given of the plants planted in pots. Of the total of 2369 plants planted in pots only 962 or 40,6 % could be fixed, a small part of the remaining 59,4 % died before they had grown up sufficiently to be fixed; the greater part was alive, but grew so slowly that they did not produce any roots for fixations during the whole summer. Of the fixed 962 plants only 617 could be 2n-determined, the fixation of the rest did not give plates clear enough for counting.

Several of these different types of elimination may be considered to be numerically selective, especially the gamete abortion and the zygotic and embryonic lethality. Among the seedlings and young plants a certain degree of the elimination is probably numerically selective, another part of this elimination can be attributed to injuries due to transplanting and so on. In Tables 8 and 5 it will be seen that only 508 of the 617 plants counted survived the first winter, i. e. a loss of 17,7 %. The loss is $11{,}1 \pm 0{,}3$ % for the 2x-plants and $19{,}0 \pm 0{,}1$ %

for the intermediate plants (Table 5) and thus the percentage of 2x-plants has increased from 16,5 to 17,8. *It therefore seems to be true that in agreement with the results of other such crosses, the non-binomial distribution of the 2n-numbers in progenies of diploid and triploid crosses is due to a numerically selective elimination of gametes, zygotes and embryos, seedlings and young plants.*

The above cited *Pyrus* crosses, with a large amount of intermediate 2n-numbers in their offspring, are considered by MOFFETT (1931; cf. DARLINGTON, 1937) to show a higher viability of 17 + 7 gametes than of others. However, as pointed out by DERMEN (1936; cf. WANSCHER, 1939), this view is wrong. Instead, the distribution agrees very nearly with the binomial distribution, which is to be expected if no numerical selection takes place. For example, only one occasional plant out of 241 had the 2x-number.

It is possible to divide the known cases of crosses between diploids and triploids into three groups according to the 2n-numbers of the offspring.

Group 1. The intermediate numbers are almost completely lacking, at least in the direction 2x—3x. The 3x—2x direction yields some intermediates but in very reduced frequencies. The highest 2n-number for this group is 12 (*Tulipa, Datura, Solanum*). To this group belongs the main part of all crosses between diploids and triploids.

Group 2. The intermediate numbers are well represented but in reduced frequencies. Reciprocal crosses give the same result. Only one case is known, *Populus tremula*, x = 19.

Group 3. The 2n-numbers of the offspring are present in binomial distribution between 2x and 3x. Reciprocal crosses give the same result. One known case only, *Pyrus malus*, x = 17.

The differences between these groups are, in fact, not qualitative but quantitative, consisting of a gradient viability of aneuploid plants. In trying to find a plausible explanation of the higher viability of aneuploids in Groups 2 and 3 a modification of DARLINGTON's view, concerning secondary polyploidy, may be attempted. Surely, the x-numbers 19 and 17 cannot be considered to be primary haploid numbers, but rather tetraploid ones, which are modified by duplication or loss of one or other chromosome, as DARLINGTON and MOFFETT (1930) have indicated for *Malus* and, of course, the primary genomes must be considered also to have been differentiated, structurally and genetically. Consequently the crosses belonging to Groups 2 and 3 are rather of a tetraploid, hexaploid type of crosses, contrary to the real (diploid, tri-

ploid) crosses of Group 1. Therefore aneuploid n- and 2n-numbers of *Populus* and *Malus* may imply smaller disturbances in the quantitative balance and more likely comprise all necessary types of chromosomes or chromosome structure than in the case of Group 1. Indeed, crosses between higher multiples in general show a higher fertility, greater frequency of intermediates, and better vitality of the aneuploids than crosses between lower multiples (MÜNTZING, 1937).

The difference between *Populus* and *Malus* may be more or less due to modifications. The progenies of *Malus* were raised under natural conditions by free or artificial pollinations. On account of the large seeds and seedlings it is easy to take good care of the offspring. The progenies of *Populus*, on the other hand, were raised from cut twigs. It is possible that this involves a greater demand on vigour for survival in both gametes and zygotes as well as embryos than under natural conditions, giving rise to the death of a greater number of subvital gametes, zygotes and embryos. Further, the seeds of *Populus* are small and very sensitive, the »struggle for life» will be relatively more severe in the germination and growth of the seedlings of *Populus* than those of *Malus*. However, there exists the possibility that real differences between *Populus*- and *Malus*-crosses occur.

In his report on meiosis of the triploid *Populus tremula*, MÜNTZING (1936) predicts the possibility of tetraploid individuals among the progeny of diploid, triploid crosses. The occurrence of a tetraploid plant in the progeny of a cross between diploid and triploid aspen has also been reported by NILSSON-EHLE (1938; cf. BERGSTRÖM, 1940). Approximately tetraploids even occur in this material, as seen in Table 5. Out of the total of 617 counted plants 18, or 2,9 %, had $\pm$ 4x chromosomes. The actual 2n-numbers have varied between $\pm$ 71 and $\pm$ 79 chromosomes, the exact tetraploid number being 76. For most of these plants the 2n-number is certainly exactly 76 (Fig. 19). But it is quite possible that some plants deviate slightly from the exact tetraploid number. That all these $\pm$ 4x-plants have originated as a result of the formation of unreduced gametes in the triploid parent is quite clear. The formation of unreduced gametes is also indicated by the studies of meiosis. That unreduced gametes need not necessarily have the exact somatic chromosome number has been pointed out by MÜNTZING (1937). All the 18 tetraploids were produced in crosses with the same triploid parent. But there are no reasons for assuming that the other two triploids used are not capable of producing tetraploids, too. These two triploids were used only to a small extent, as compared with the one pro-

ducing tetraploids, and only 3 out of 16 crosses with the latter produced tetraploids. The frequency of ± 4x-plants in the three crosses, having produced tetraploids, is very variable. In Cross 62 it is 1 tetraploid out of 99 plants, in Cross 203 it is 4 out of 30, and in Cross 200 it is 13 out of 20 plants. Such a variable frequency from 0 to a high value is also to be expected in view of the observation that an occasional loculus of the stamens produced large pollen grains in a very high frequency when other loculi produced pollen grains of normal size exclusively. Therefore the number of tetraploids may be correlated to the amount of the pollen grains used, derived from such exceptional loculi.

VII. STOMATA LENGTH AND PLANT HEIGHT OF THE PROGENY.

1. STOMATA LENGTH.

In Table 9 the stomata lengths are given for the aneuploid offspring of the 2x—3x cross No. 257. The measurements were made at

TABLE 9. *Stomata-length and chromosome number of the aneuploid offspring of Cross 257, 2x—3x aspen.*

Chromosome number	38	39	40	41	42	43	44	45	46	47	48
Length of stomata ...	13,3	12,6	11,4	11,8	12,4	11,8	12,9	13,4	12,6	12,9	13,3
Chromosome number	49	50	51	52	53	54	55	56	Mean		
Length of stomata ...	13,8	13,2	13,9	11,9	13,0	11,7	12,5	14,5	12,8 ± 0,19		

the end of the second growing season. As a rule three plants of each 2n-number were measured and the mean calculated. In a few cases only two or even one plant was available for measurement. Of each plant 50 stomata of one leaf were measured — one unit being 2,3 μ. As seen, there is no connection between stomata length and 2n-number. The regression of stomata lengths on 2n-numbers, calculated according to FISHER's formula, is 0,05 with $p > 0,9$. The average stomata lengths of this aneuploid variation is 12,8 ± 0,19 units. Table 10 gives, for comparison, the mean stomata lengths of two pure diploid crosses, the aneuploid cross No. 257 of Table 8 and of the 2x—3x cross No. 200, distinguished by a large frequency of ± 4x-plants. No doubt, in spite of the non-

existent regression of stomata lengths on 2n-numbers within the aneuploid progeny, the mean stomata length of this variation, having the mean 2n-number 47, is significatively higher than the mean stomata length of the two diploid crosses. Furthermore, the mean stomata length of the ± 4x-plants is higher than the comparable values of the diploids as well as of the aneuploids. Thus the fact that the 2n-number is a factor influencing the stomata length (cell size), established by the treatment of diploids and triploids, also applies to aneuploids and tetraploids. And, as in the case of the diploids and

TABLE 10. *Stomata-length and chromosome number for diploid, aneuploid and tetraploid aspen.*

Cross number	301	276	257	200		
Chromosome number	38	38	38—56	38	50	± 4x
Number of individuals	5	23	49	2	1	11
Stomata-length ...	$11{,}6 \pm 0{,}10$	$11{,}8 \pm 0{,}15$	$12{,}8 \pm 0{,}19$	$11{,}8 \pm 1{,}25$	13,0	$15{,}3 \pm 0{,}31$

triploids, other factors also influence the stomata length of aneuploids and tetraploids. This is especially marked in the aneuploids, within which there is no regression of stomata length on 2n-numbers. Another factor operating here may be the chromosome components of a given 2n-number. A certain 2n-number may be built up predominantly by small or by large chromosomes, and that may influence the cell size. Further, genes may be assumed to exist, which influence the cell size, and these genes will be included in various ways with different combinations of the chromosomes.

2. PLANT HEIGHT.

The vigour of the 2x—3x offspring is very variable at the end of the second growing season. This variation is as great within as between the 2n-numbers. On an average the plants are poor (cf. BERGSTRÖM, 1940). In Table 11 measurements of plant height are tabulated for each 2n-number. The standard errors are great in agreement with the great variation in vigour among plants with the same 2n-number. The only conclusions to be drawn are that plants with 38 and 39 chromosomes are much taller than plants with any other 2n-numbers. The reason why the height of the 39-plants is so great is because some 38-plants may also have been included in this category owing to the

limited accuracy of the 2n-determinations. In general, plants having aneuploid 2n-numbers are inferior in vigour to diploid plants, as discussed by MÜNTZING (1937). One example quite comparable with the 2x—3x aspen offspring is given by MÜNTZING (1937) in the cross between the diploid (2n = 14) *Dactylis Aschersoniana* and the triploid hybrid between this species and its tetraploid derivative *D. glomerata*. In this cross one or a few plants of all intermediate 2n-numbers were realized. All the intermediate plants were much inferior in vigour as compared with the diploid *D. Aschersoniana*.

TABLE 11. *Chromosome numbers and plant heights after the second growing season in offspring of crosses 2x × 3x P. tremula.*

Chromosome number	Mean height cm.	Max. height cm.	Number of plants	Chromosome number	Mean height cm.	Max. height cm.	Number of plants
38	41,80 ± 3,16	139	84	50	22,21 ± 2,46	70	38
39	45,35 ± 5,77	86	17	51	25,88 ± 2,86	94	40
40	23,33 ± 2,31	44	15	52	24,67 ± 2,93	55	21
41	25,00 ± 4,02	58	15	53	33,65 ± 3,99	77	23
42	28,00 ± 4,41	80	16	54	24,54 ± 4,72	61	13
43	23,31 ± 4,35	66	14	55	25,15 ± 3,14	89	13
44	18,45 ± 3,02	40	11	56	32,83 ± 9,23	68	6
45	23,33 ± 3,41	50	15	57	33,20 ± 11,40	71	5
46	20,50 ± 2,20	61	16	•			
47	24,14 ± 2,47	63	22	•			
48	24,15 ± 2,94	97	39	•			
49	17,37 ± 2,68	54	24	± 4x ...	26,75 ± 3,74	57	16

A perhaps more surprising result is that the ± 4x-plants do not seem to be superior to the aneuploids in plant height. Here, however, another factor is involved. In Table 12 the mean plant heights of some progenies of pure diploid crosses are given. There are considerable differences in plant height of different crosses. The main source of these differences may be the occurrence of photoperiodic races within *Populus tremula*, which are very highly adapted to the special light climate of their habitats (SYLVÉN, unpubl.). All progenies have been cultivated at the institute at Svalöf, Skåne, South Sweden, at about 55° 42′ north latitude. Here the progenies of parents from North Sweden, as is the case of cross No. 204, develop very slowly. The same is to a high degree true of crosses between one southern and one northern parent, as exemplified by cross No. 301. Only crosses between

two southern aspens grow normally, crosses Nos. 251 and 288. With regard to the aneuploids it has been shown (Table 5) that the distribution of 2n-numbers is about the same for all crosses. Consequently, southern, northern and southern × northern crosses have contributed, on an average, equally to every 2n-number. Thus the means are comparable. But in the case of ± 4x-plants, 15 out of 16 measured plants occasionally belong to the Crosses 200 and 203, both of which have the triploid male aspen from Lillö at about 55° 42′ north latitude as fathers, and as mother parents two different diploid females from Vittjärv in Norrbotten at about 66° north latitude, not far from the arctic circle.

TABLE 12. *Plant-heights after the second growing season of offspring of some 2x-crosses.*

Cross number	Combination	Mean height cm.	Max. height cm.	Number of plants
251	Belgium × Skåne	71,00 ± 4,84	125	20
276	Västergötland × Skåne	46,57 ± 3,20	91	42
301	Skåne × Medelpad	33,67 ± 2,79	99	45
201	Medelpad × Medelpad	37,47 ± 3,55	60	19
204	Norrbotten × Medelpad	20,84 ± 2,39	71	44
288	*P. alba* (Skåne) × Skåne	86,06 ± 8,90	168	18
286	*P. alba* (Skåne) × Norrbotten	42,47 ± 4,93	77	19

Consequently, the plant height of the ± 4x-plants is not comparable with the corresponding values for the other 2n-numbers. Further, nothing can be said as to the absolute vigour of tetraploid aspen, owing to the fact that the tetraploids, available at present, are not photoperiodically adapted to the actual light condition occurring at Svalöf.

VIII. SEX OF THE TETRAPLOIDS.

Of course, it has not yet been possible to determine the sex of the tetraploid seedlings produced. By means of grafting buds in the crowns of old fruitful aspens it will, however, be possible to determine the sex in the near future. In spite of this some conjectures as to the possible sex may be justified. According to the assumptions, made in the discussion of the sex of the triploids, the triploid male parents may have the constitution XXY. Then the diploid female must be XX. All the discussed tetraploids have originated as a result of unreduced

triploid male gametes, i. e. XXY-gametes. Therefore only one sort of tetraploids will result, viz. XXXY individuals. These may be males, females or intersexes. In *Melandrium*, WARMKE and BLAKESLEE (1939) found the 4A + XXXY individuals to be pure male in 65 cases and intersexes in 3 cases. If, on the other hand, the triploid male is of the constitution XXX, the diploid female must be XY. Then the tetraploids will be of two constitutions, viz. XXXX and XXXY. Both these constitutions may phenotypically be males, or the one male and the other female or intersex. Thus if the two tetraploid constitutions XXXX and XXXY are phenotypically different, the sexes of the tetraploids — in any case if tetraploids, derived from female unreduced gametes, are also available — will offer an opportunity to prove whether the male or the female represents the heterozygous sex in diploid *P. tremula*. If, on the contrary, both the constitutions have the same phenotype, nothing can be said about the sex determination. And if both constitutions are of the same sex or if one of them is a sexually non-functional intersex, it would be impossible for the tetraploids to maintain themselves by sexual reproduction. However, a somatic doubling of the diploid heterozygous sex would give tetraploids of the constitution XXYY, which would probably be of the same sex as XY. Tetraploid aspens originating by somatic doubling have been experimentally produced by means of colchicine treatment of germinating seeds (unpubl.).

IX. DISCUSSION.

The amentiferous plants are in general considered to be primitive and of ancient origin. *Salicaceæ*, comprising *Salix* and *Populus*, is the most primitive family. In this group the x-number is 19. Other members of *Amentiferæ* have x-numbers 14 (*Alnus*, *Betula*), 8 (*Carpinus*), 12 (*Fagus*, *Quercus*, *Castanea*), 16 (*Juglans*) (TISCHLER, 1926, 1931, 1936, 1938). The x-number 19 in *Populus* and *Salix* cannot be considered to be a primary one. In *Pyrus* the x-number 17 is considered to be derived from a primary x-number of 7. This conclusion is drawn mainly from the evidence of »secondary pairing» (DARLINGTON and MOFFETT, 1930). Also in other species secondary pairing has been used as evidence of genome complexity (cf. DARLINGTON, 1937). In *Populus* and *Salix* secondary pairing has never been reported. MOFFETT's view that plants with 34 + 7 chromosomes in the progeny of crosses between diploid and triploid apples are more viable than other individuals (MOFFETT, 1931) has been shown to be wrong by DERMEN (1936;

cf. WANSCHER, 1939). But in spite of this the assumption of a genome complexity affords a possibility of explaining the high viability of aneuploid apples. In this respect *Populus tremula* behaves intermediately between *Pyrus malus* and other species. Thus it seems as if the complex genome of *Populus* has lost almost all features of its origin, and on the whole behaves as a primary one. The only evidence is the rather high viability of aneuploids. This may indicate a more ancient origin than that of *Pyrus,* in agreement with the view of most taxonomists, that *Populus* belongs to an old group of plants. Both *Populus* and *Salix* are dioecious. Heterochromosomes have been reported for several species, but at least as far as *P. tremula* is concerned this report is doubtful. Polyploidy has been of a very different importance in the two genera. In *Salix* polyploidy is common, diploid, tetraploid and hexaploid species are reported (BLACKBURN and HARRISON, 1924). In *Populus* no polyploid species is known for certain. In spite of this, triploid clones of *P. tremula* are fairly common. In *P. alba* and *P. canescens* — probably a hybrid between *P. tremula* and *P. alba* — triploids have been reported (PETO, 1938). These triploids have probably originated due to unreduced gametes (MÜNTZING, 1936; PETO, 1938). The occurrence of extremely large — presumably unreduced — pollen grains by diploid *Populus* species has been found by PETO (1938). The triploids, too, produce »giant» pollen grains, as shown by MÜNTZING (1936) and PETO (1938). In artificial crossings between a diploid female and a triploid male *P. tremula* about 3 % of the offspring were found to be tetraploids. Thus there is a great possibility of intraspecific tetraploids arising in nature. Interspecific hybrids within *Populus* are common in nature (HOUTZAGERS, 1937). One diploid hybrid has been shown to produce large pollen grains and one hybrid to be a triploid, producing large pollen grains (PETO, 1938). For that reason there is a great possibility also of interspecific polyploids occurring in nature. Thus the lack of tetraploid and higher polyploid *Populus* species and races must be attributed to special factors. The fact may be that eventually arising tetraploids for some reason or other cannot survive in the competition in nature. Another factor well worth mentioning is the dioecy. Of course a single tetraploid, if purely dioecious, cannot maintain itself by sexual reproduction. The condition for this must be that two tetraploids of opposite sexes arise in the neighbourhood of each other. Such events may be statistically rare, but, no doubt, the natural population has in the course of time been large enough to create them. And in the related genus *Salix* polyploidy

is common in spite of dioecy. Furthermore, some *Populus* species — for instance, *P. tremula* — are capable of vegetative propagation in nature by means of suckers, and for that reason these species have an ability of migrating over large areas. Thus the questions concerning the absent natural polyploidy in *Populus* are at present impossible to answer. Further experiments may perhaps provide the clue.

SUMMARY.

1. Some new triploid *Populus tremula* clones in Sweden have been found. Altogether nine triploid clones are known.

2. Data are given, showing the connection between leaf size, lengths of stomata and 2n-numbers. It is pointed out that large leaves are always correlated to long stomata, but not always to triploidy.

3. The pollen properties of the triploids, compared with those of the diploids, have been investigated. The triploids have larger pollen grains and poorer pollen than the diploids, but the diploid and triploid variation overlaps.

4. Among the triploids pure males and pure females exist. The type of sex determination is discussed.

5. Meiosis of diploid male aspens has been studied. In general, diploid meiosis is quite regular. Four diploid clones out of sixteen have shown a varying frequency of univalents. The behaviour of the univalents is described.

6. Meiosis in triploids follows the general course described by MÜNTZING (1936). Some further results are added.

7. Crosses between diploid and triploid aspens have been produced in both directions.

8. The 2n-numbers of 617 offspring plants are given. Intermediate 2n-numbers between 2x and 3x are represented in a high frequency, but the distribution is not binomial. Reciprocal crosses give the same result. In the progeny 18 $\pm$ 4x-plants were found. The occurrence of tetraploids is attributed to the formation of unreduced gametes in the triploid parent.

9. The deviation of the progeny from the binomial distribution is attributed to numerically selective elimination. It has been shown that this elimination involves gametes, zygotes and embryos as well as seedlings and young plants. The elimination of gametes as well as zygotes and embryos is approximately identical in reciprocal crosses.

10. Measurements of stomata and plant-height of the progeny are

given. No correlation between stomata-length and 2n-number within the 2x—3x variation was found, but on an average the aneuploids had longer stomata than the diploids. The tetraploids had longer stomata than both aneuploids and diploids. Plant-height is very variable among the aneuploids. The aneuploids are poorer than the diploids. No conclusions can be drawn as to the vigour of the tetraploids, because of the fact that photoperiodic races exist within *P. tremula* (SYLVÉN, unpubl.), and the tetraploids are occasionally produced by crosses which do not give progenies, adapted to the light climate of the experimental station.

11. The possible sex of the tetraploids is discussed.

12. The x-number and the lack of polyploidy within the genus *Populus* is discussed.

Acknowledgments. — I wish to express my sincere gratitude to Professor H. NILSSON-EHLE, the initiator of this investigation, to Dr. N. SYLVÉN, the head of the institute, and to the staff of the laboratory and nurseries, without whose careful labour this work would have been impossible. Further, I am indepted to Dr. Å. GUSTAFSSON for reading of the manuscript.

LITERATURE CITED.

1. BEADLE, G. W. and MC CLINTOCK, B. 1928. A genic disturbance of meiosis in *Zea mays*. — Science LXVIII.
2. BELLING, J. and BLAKESLEE, A. F. 1922. The assortment of chromosomes in triploid *Datura*. — Amer. Nat. 56.
3. BERGNER, A. D., CARTLEDGE, J. L. and BLAKESLEE, A. F. 1934. Chromosome behaviour due to a gene, which prevents metaphase pairing in *Datura*. — Cytologia 6.
4. BERGSTRÖM, I. 1940. On the progeny of diploid × triploid *Populus tremula*. With special reference to the occurrence of tetraploidy. — Hereditas XXVI.
5. BLACKBURN, K. B. and HARRISON, J. N. 1924. A preliminary account of the chromosomes and chromosome behaviour in the *Salicaceæ*. — Ann. Bot. Vol. XXXVIII.
6. BLOMQUIST, S. G:SON. 1937. Ett fynd av jätteasp (*Populus tremula gigas*) i Medelpad. — Bot. Not.
7. DARLINGTON, C. D. 1937. Recent advances in cytology. 2nd ed. — London.
8. DARLINGTON, C. D. and MOFFETT, A. A. 1930. Primary and secondary chromosome balance in *Pyrus*. — Journ. of Gen. Vol. 22.
9. DERMEN, H. 1936. Fertilization in the Baldwin apple, a triploid variety. — Journ. of the Arnold Arboretum. Vol. 17.
10. FISHER, R. A. 1938. Statistical methods for research workers. 7th ed. — London.

11. HOUTZAGERS, G. 1937. Het geslacht *Populus* in verband met zijn beteekenis voor de houttielt. — Wageningen.
12. MATHER, K. 1935. Chromosome behaviour in a triploid wheat hybrid. — Zschr. f. Zellf. u. mikr. Anat. 23.
13. MEURMAN, O. 1925. The chromosome behaviour of some dioecious plants and their relatives with special reference to the sex chromosomes. — Comm. Biol. Soc. Sci. Fenn. 2.
14. MOFFETT, A. A. 1931. The chromosome constitution of the *Pomoides*. — Proc. of Roy. Soc. B. Vol. 108.
15. MÜNTZING, A. 1936. The chromosomes of a giant *Populus tremula*. — Hereditas XXI.
16. — 1937. The effects of chromosomal variation in *Dactylis*. — Hereditas XXIII.
17. NEBEL, B. R. 1933. Chromosome numbers in aneuploid apple seedlings. — N. York St. Agr. Exp. St. Bull. 109.
18. NILSSON-EHLE, H. 1936. Über eine in der Natur gefundene Gigasform von *Populus tremula*. — Hereditas XXI.
19. — 1938. Framställning av skogsträd med ökat kromosomtal och ökad virkesproduktion. — Svensk Papperstidning.
20. PETO, F. H. 1938. Cytology of poplar species and natural hybrids. — Can. Journ. Res. C. 16.
21. STRAUB, J. 1937. Die Wirkung von Temperaturstözen auf die Reduktionsteilung. — Ber. d. deutsch. bot. Ges. LV.
22. TISCHLER, G. 1926, 1931, 1935/36, 1938. Pflanzliche Chromosomen-Zahlen. — Tabulæ biologicæ periodicæ. Bd III, VII, XI/XII, XVI.
23. TOMETORP, G. 1937. The chromosome numbers of two new giant *Populus tremula*. — Bot. Not.
24. UPCOTT, M. and PHILP, J. 1939. The genetic structure of *Tulipa*. IV. Balance, selection and fertility. — Journ. of Gen. 38.
25. WANSCHER, J. H. 1939. Contribution to the cytology and life history of apple and pear. — Den kong. Vet. og Landbohøjskoles Aarsskrift 1939. København.
26. WARMKE, H. E. and BLAKESLEE, A. F. 1939. Sex mechanism in polyploids of *Melandrium*. — Science 89.
27. WETTSTEIN, W. v. 1937. Forstpflanzliche Züchtungsversuche besonders mit *Populus*. — Bot. Not.

CONTENTS.

I. Introduction 321
II. Material and methods 322
III. Triploid aspen 323
1. Occurrence 323
2. Cell size 323
3. Pollen properties 324
4. Sex 326

IV. Meiosis of diploid aspen 327
V. Meiosis of triploid aspen 331
VI. Crosses between diploid and triploid aspen 332
VII. Stomata length and plant height of the progeny 343
1. Stomata length 343
2. Plant height 344
VIII. Sex of the tetraploids 346
IX. Discussion 347
Summary 349
Literature cited 350

THE CYTOLOGY OF ALLIUM AMPLECTENS AND THE OCCURRENCE IN NATURE OF ITS ASYNAPSIS

BY *ALBERT LEVAN*
CYTO-GENETIC LABORATORY, SVALÖF, SWEDEN

INTRODUCTION.

MOST of the *Allium* species occurring in the Pacific Coast region of North America are endemic and closely related. They form a morphological type readily distinguishable from the *Allium* species of the Old World, which exhibit a decidedly greater diversity. The relative uniformity of the American *Allium* species is reflected in their chromosome conditions: all West-American species so far studied have the basic number 7, while the Eur-Asiatic species usually have the number 8, although the numbers 7 and 9 are also found among them. Unfortunately the taxonomy of the genus *Allium* is not sufficiently known to allow any conclusions concerning any possible relationship of the American species to any certain group of the Eur-Asiatic species. A study along these lines should give important results, among others, in phylogenetic and plant-geographical respects.

In order to prepare the ground for a joint attack on the taxonomy, cytology and species formation of the genus *Allium*, I have for some years examined different small groups of species from a cytological and in certain especially favourable cases also phylogenetic view-point. *Allium* must be regarded as an ideal material for such studies. About 300 species are distributed all over the northern hemisphere. They are characterized by remarkably well-defined and constant cytological features. The idiograms of the species may be studied with an almost schematic clearness during the first microspore mitosis. Several species may be recognized only by the appearance of their satellited chromosome. And cytologically observable variation, both structural and numerical, very often gives the key to the type formation within related groups of taxonomic units.

In the present paper I take up for examination a West-American species, *Allium amplectens* TORR. This species is of especially great interest because of a genetic abnormality of meiosis, present in certain

forms of the species. Such gene-controlled deviations from the normal course of meiosis are by no means rare in *Allium*. On several earlier occasions I directed attention to the formation of dyad pollen due to failure of the second meiotic division, an abnormality now and then found in most *Allium* species. In *Allium amplectens*, on the other hand, asynapsis leads to the complete failure of the first meiotic division, a state of things not previously encountered in *Allium*.

The outlines of this asynapsis process have already been published (LEVAN, 1938). The process differs in certain fundamental respects from all cases of asynapsis previously described. Its main characteristic is its completeness, which is associated with great regularity in the asynaptic meiosis. In fact, the asynaptic meiosis takes place with fewer disturbances than meiosis in normal synaptic plants of the same species, which suffer from the usual disturbances due to autopolyploidy. The pollen formed after the asynaptic meiosis has a regular shape, deviating from normal *Allium* pollen. This permits a rapid diagnosing of asynapsis even in dried material. On account of this very favourable condition, the investigation of the cytology of the species could be completed by a plant-geographical study.

In the first part of this paper the cytology of normal and asynaptic *Allium amplectens* will be treated. The material of this part consists of a few forms of the species which I brought home from California. They turned out to include samples of both types of meiosis. The second part of the paper is based on herbarium material from different parts of the distribution area of the species. The asynapsis, studied in detail in the first part, can now be placed in its plant-geographical connexion.

This investigation has been assisted in many ways by several institutions and persons, whom I wish to mention here, and at the same time express to them my sincere gratitude. My studies in California were made possible by grants from two Swedish institutions: Sverige—Amerika Stiftelsen, Stockholm, and Kungliga och Hvitfeldtska Stipendieinrättningen, Gothenburg. Drs. JENS CLAUSEN and DAVID D. KECK gave me the opportunity of collecting living material of *Allium amplectens* during an excursion from Stanford University. Dr. G. LEDYARD STEBBINS Jr. procured living material, among others, of *Allium serratum*, mentioned below. Miss ALICE EASTWOOD, Drs. JOHN T. HOWELL, DAVID D. KECK, and HERBERT L. MASON examined the distribution map of *Allium amplectens* and suggested important improvements. The Curators of the herbaria of the University of California, Berkeley,

California Academy of Science, San Francisco, and Stanford University lent me dried material of the species. Dr. CARL W. SHARSMITH, Curator of the herbarium, State College of Washington, Pullman, and the Librarian, Bureau of Plant Industry, Department of Agriculture, Washington, D. C., helped me in various ways. Miss ANNA NORDSTRAND, Hilleshög, has given me valuable technical assistance.

I. CYTOLOGICAL STUDIES.

1. MATERIAL AND METHODS.

The material for the cytological study of *Allium amplectens* was collected on an excursion on the 13th May, 1937, in Santa Clara County, California. Six different forms were collected. They are listed below together with Dr. KECK's description of the localities:

7043. (KECK No. 4531). Mt. Hamilton — San Antonio Valley road, at Isabel Creek, just east of crossing, in creeklet. Bulbs in mud. No plants in neighbouring slopes.

7042. (KECK No. 4537). Arroyo Bayo, 5 miles east of Isabel Creek, Mt. Hamilton range. In alluvial flats and in creek bed.

7048. (KECK No. 4540). Arroyo Bayo, 6,8 miles south-east of Isabel Creek, in moist creek bed.

7049—7051. (KECK Nos. 4549—4551). Beauregard Creek, 14,2 miles from crossing of Isabel Creek (31 miles from Livermore). In dry soil.

Among these forms, 7043, 7049 and 7051 turned out to be asynaptic and triploid, while the rest had normal meiosis and were tetraploid. Another normal tetraploid of *Allium amplectens* (my No. 657) was investigated earlier (LEVAN, 1931). It had been procured from the Botanic Garden of Copenhagen.

Since these forms of *Allium amplectens* cytologically represent two types, the normal and the asynaptic, no distinction will subsequently be made between the different forms within each type. I wish to point out, however, that numerous slides from different fixations have been studied of all forms. They all showed good agreement in their main cytological features. It may therefore be concluded that the asynapsis is not due to modificative influences or mere chance occurrences.

The general appearance of two of the investigated forms is seen in Fig. 1 *b—c*, which represent one triploid asynaptic form and one tetraploid normal form. Fig. 1 *a* is a related diploid species, *Allium*

serratum, collected in the same region as *Allium amplectens*. A close picture of the *amplectens* flowers is shown in Fig. 10.

The cytological methods employed are the same as those previously described (LEVAN, 1932, 1936), viz. meiosis fixed in whole flower buds in NAVASHIN or in smears in BENDA—GEITLER, pollen fixed in smears in BOUIN—ALLEN. The drawings were made with the aid of the following lens system: ZEISS apochromatic objective H 120 × 30 or in general

Fig. 1 *a*: *Allium serratum*, *b*: *Allium amplectens*, triploid form, *c*: ditto, tetraploid form.

views × 15, which on drawing gives a magnification of 6500 and 3250 times respectively.

2. SOMATIC CHROMOSOMES.

The basic number of *Allium amplectens* is 7, as already mentioned. Diploid forms with the somatic number 14 occur probably in nature (see Chapter II: 3), but no such forms were found among the living material available for cytological study. The forms cultivated by me

were triploids and tetraploids with the somatic numbers 21 and 28 respectively.

All these forms agree in their chromosome morphology. The chromosomes are all of one type, two-armed with medially or submedially located centromeres (Fig. 7). Satellites are entirely missing. And since even very small satellites are easily seen in the pollen mitoses of related species, for instance *Allium serratum* (Fig. 9 *a*, *b*), it is highly probable that *Allium amplectens* is really devoid of satellites. The very peculiar nucleolar conditions present in *Allium amplectens* (cf. Chapter I: 6) are presumably connected with this absence of satellites.

The chromosome size is very large, even compared with other *Allium* species. The longest chromosomes of the idiogram have during the pollen metaphase a length of up to 15 μ, while their breadth in the neighbourhood of the centromeric constriction is somewhat less than 1 μ. It has been previously shown (LEVAN, 1935 a) among other *Allium* species that members of the 7-series have on an average longer chromosomes than 8-chromosome species. While the average length of the former was estimated at 13 μ, the latter had a length of about 9 μ. The chromosomes of *Allium amplectens* are exceeded in size only by another American species, viz. *Allium* (*Nothoscordum*) *fragrans*.

3. MEIOSIS OF THE NORMAL TYPE.

Meiosis of all the tetraploids investigated takes place without any deviations from the normal course of meiosis already studied in several autotetraploid *Allium* species. Thus it agrees completely with meiosis of the only other known tetraploid American species, *Allium validum*. All stages of meiosis could be studied with great clearness. Already at pachytene very evident quadrivalents were observed (Fig. 2 *a*—*c*). The pairing was very good, the unpaired threads frequently met with could often be seen to be due to exchanges of partners within the quadrivalents. Due to the large size of the chromosomes and chromomeres the differences in shape and size of individual chromomeres could easily be demonstrated, in the same manner as BELLING did (1928) in *Lilium*. It was found that the paired chromomeres of homologous threads were usually identical in shape. Very striking exceptions from this rule were not so seldom met with, however, and it was noticed that within regions where dissimilarities in appearance between the paired chromomeres were present, the pairing seemed to be loosened, loops of unpaired threads being formed. An instance of this is pictured in Fig. 2 *d*.

Both the shape and number of chromomeres within the pictured region are quite different in the two threads.

In diplotene normal chiasmata are seen (Fig 2 *e—g*), some chiasmata holding the chromosomes together into quadrivalents. Other quadrivalents separate, due to lack of chiasmata, into 2 bivalents or one

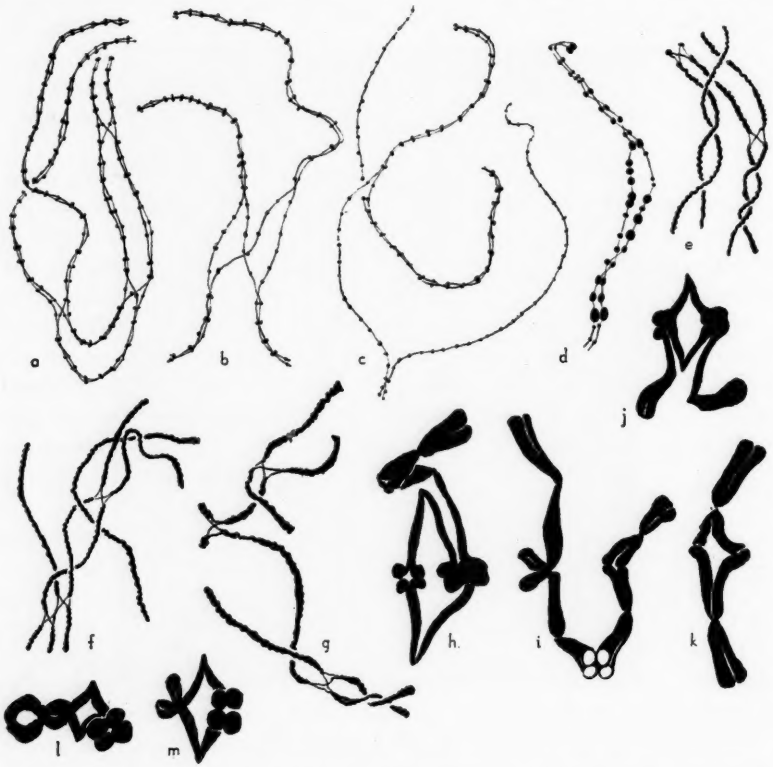


Fig. 2. Meiosis of the normal type, *a—d*: pachytene, *e—g*: diplotene, *h—m*: metaphase I. — × 3250 (except *d*, which is a free hand drawing).

trivalent and one univalent (as in Fig. 2 *e* and *f*). At diakinesis and metaphase I the chromosomes assume the typical meiotic appearance. It is now possible to analyse whole cells, and it may then be seen that one or two quadrivalents occur in almost every cell. In single cells all the chromosomes may remain associated into 7 quadrivalents. Fig. 2 *h—j* shows a couple of the most common types of quadrivalents.

Chains of four were commonest, and single rings and frying-pan quadrivalents occurred somewhat more rarely. The bivalents (Fig. 2 *k*—*m*) might have 1—5 chiasmata, commonly 1—2.

At the first anaphase very often a number of univalents were present. In one slide the following frequency of univalents was counted:

Number of univalents:	0	1	2	3	4	Total	Med.
Number of cases:	4	7	12	6	5	34	2,0

The univalents always formed a secondary equatorial plate and were eventually divided in the first division. This causes a certain disturbance of the first division, resulting in the formation of micronuclei in the interkinesis. The following number of micronuclei was determined in one slide:

Number of micronuclei:	0	1	2	3	4	5	Total	Med.
Number of cases:	6	10	14	8	4	1	43	1,9

These irregularities of meiosis give rise to a somewhat varying number of chromosomes in the pollen grains.

4. MEIOSIS OF THE ASYNAPTIC TYPE.

The zygotene pairing of the asynaptic type takes place absolutely normally in the manner previously described in synaptic triploid *Allium* species, e. g. *Allium Schoenoprasum* and *carinatum*. Probably 7 trivalents are formed in most cells. Trivalents which I was able to analyse at pachytene usually had 2 pairing blocks (Fig. 3 *a*—*d*). I wish especially to emphasize that the chromomere paring within the frequently very clearly visible pachytene configurations was, as far as could be seen, of the same strong and intimate nature as is found in the synaptic types. Thus, it is impossible at this stage to distinguish the asynaptic type from normal types with complete pairing.

Already at early diplotene, however, a striking difference from the normal type is observed. All points of crossings within the trivalents now turn out to consist of mere overlappings and no real chiasmata at all are found. It is evident that the formation of chiasmata has failed

in some way or other. That the three chromosomes of the trivalents are still placed together (Fig. 3 *e—j*) is due to their relational spiralis-

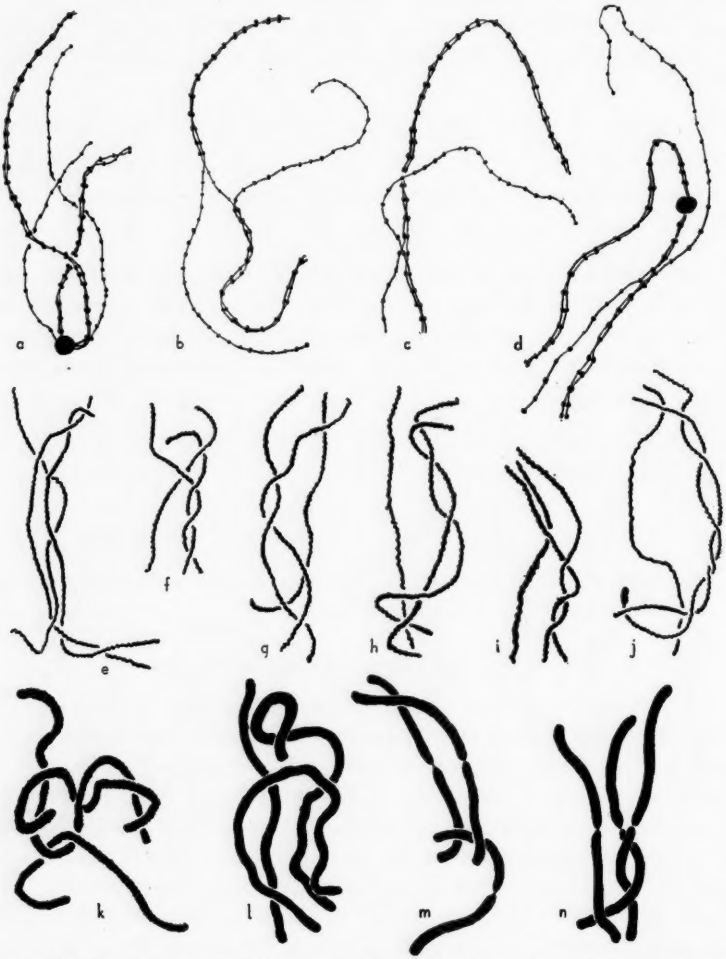


Fig. 3. Meiosis of the asynaptic type, *a—d*: pachytene, *e—j*: diplotene, *k—n*: diakinesis. — × 3250.

ation. Because of this it is often possible, even at late diplotene and diakinesis, to recognize the original pairing blocks (Fig. 3 *k*, *l*).

At the same time as the contraction of the chromosomes increases,

the relational spirals uncoil and numerous free univalents begin to appear. Their position, however, indicates for a long time which chromosomes originate from the same trivalent. At diakinesis (Fig. 3 *m—n*, Fig. 4 *a—c*) the centromeric region of the chromosomes may be clearly discerned as a constriction medially located in all chromosomes. The chromosomes gradually acquire a more somatic metaphase appearance,

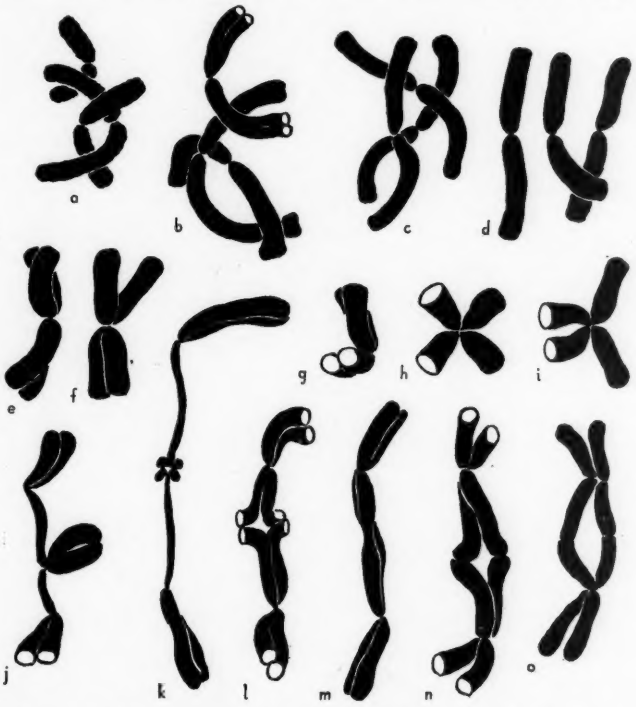


Fig. 4. Meiosis of the asynaptic type, *a—c*: diakinesis, *d*: metaphase I, *e—i*: the splitting of the metaphase I univalents, *j—o*: bivalents of the asynaptic type, seen in polar view. — × 3250.

and when the nuclear membrane disappears they resemble ordinary root chromosomes, even if their contraction is somewhat stronger. The split between the daughter chromatids, which was quite apparent during diplotene and diakinesis, has by now become invisible (Fig. 4 *d*).

Here ceases, however, the resemblance to somatic chromosomes, in which, at this stage, the centromeres would very soon have divided. In the asynaptic metaphase, on the other hand, the centromeres

remain undivided. The chromosomes are arranged with their longitudinal direction extended in the polar plane and proceed towards the equator (Fig. 6 *b*). There is usually not space enough for all 21 univalents in one equatorial plate, so 4 or 5 chromosomes form accessory plates on one or both sides of the equator. During this process the daughter chromatids begin to fall apart. Since the centromeres are still single, however, each chromosome assumes a more or less typical cross-shape (Fig. 4 *e—i*, Fig. 6 *c*).

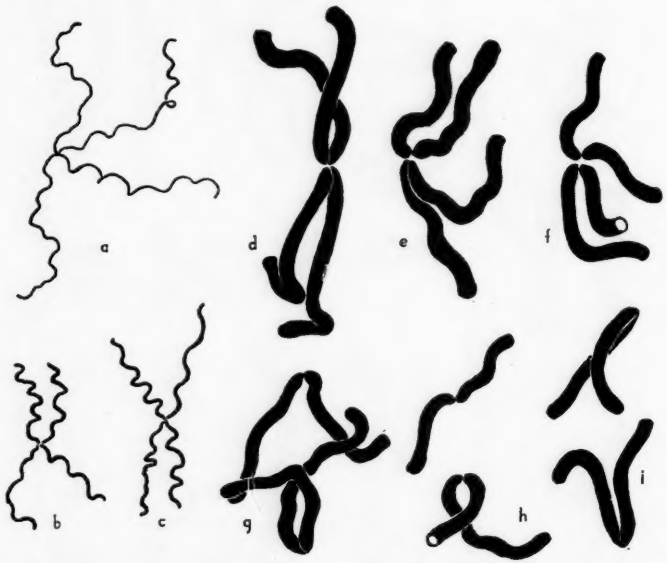


Fig. 5. Meiosis of the asynaptic type, *a—c*: univalents at interkinesis, *d—f*: second metaphase, *g—i*: second anaphase. — × 3250.

A great number of slides of this stage was examined, and I then found that a chiasma could really be formed now and then. The frequency of these chiasmata varied and was always very low. In most slides one chiasma could be found in some 500 cells, in other slides one chiasma in 100 cells and in one slide even 4 chiasmata in 100 cells. These chiasmata gave rise to bivalents and in 2 observed cases to trivalents. Considering the extremely low frequency of chiasmata it is impossible that a random distribution of chiasmata should bring about any formation of trivalents. The 2 chiasmata within the observed trivalents must consequently be due to the same cause, presumably a

change in the precocity conditions within the three chromosomes of the pachytene trivalent.

The studied bivalents, some of which are pictured in Fig. 4 *j*—*o*, all had one chiasma each. This chiasma was in most cases wholly terminalised, but in a few cases an interstitial position of the chiasma was observed. In one case it was situated about half-ways out on the chromosome arms (Fig. 4 *j*). The fact that the chiasmata of the bivalents were usually terminal suggests that the repulsion between the centromeres of the bivalents was of normal effect. Also the shape of the bivalents indicated the same thing: the portion of the bivalent lying between the chiasma and the centromeres was very often extended into a narrow thread (Fig. 4 *k*).

This condition is perhaps somewhat surprising, since the orientation of the bivalents in relation to the poles was always quite different from the normal bivalent orientation. In no case were the centromeres of the bivalents observed to be co-orientated, on the contrary, they were always situated within the equatorial plate at the same level as the centromeres of the univalents. And it could happen that the arm connecting the two centromeres of the bivalent ran right through the whole plate.

This condition has a certain significance for the understanding of the mechanics of the centromeres. Evidently it is not the chiasma formation *per se* that causes the characteristic orientation of the meiotic bivalents, rather it must be some quality within the centromeres themselves. It may be concluded in accordance with DARLINGTON (1937) that a polarisation of the centromeres brings about a predisposition to auto-orientation, while a lacking of polarisation causes co-orientation. And this polarisation has apparently nothing to do with the presence or absence of chiasmata. In the solitary bivalents of the asynaptic meiosis the orientation is the same as that found earlier in the so-called somatic bivalents, originated by segmental interchange between different somatic chromosomes. The further bearing of this behaviour of the centromeres on the interpretation of the asynapsis of *Allium amplectens* will be discussed in the final chapter.

After some time the chromosomes begin to exhibit a decreased stainability and an evident despiralisation, in fact, they begin to show telophase characters. Sometimes it may appear as if the centromeres had divided, the daughter chromatids lying almost parallelly. This is, however, a false appearance due to the fact that the region of the chromosomes close to the centromeres loses its stain somewhat earlier

than the rest of the chromosomes. The subsequent stages show clearly that the centromeres remain undivided all through interkinesis. The nuclear membrane begins to develop, sometimes separately around each chromosome, usually, however, around the whole chromosome group. The chromosomes of the periphery very often form nuclear lobes of their own, communicating with the large central part of the nucleus. The large single telophase nucleus formed in each pollen mother cell has at first a peculiar disciform shape, due to the fact that the chromosomes

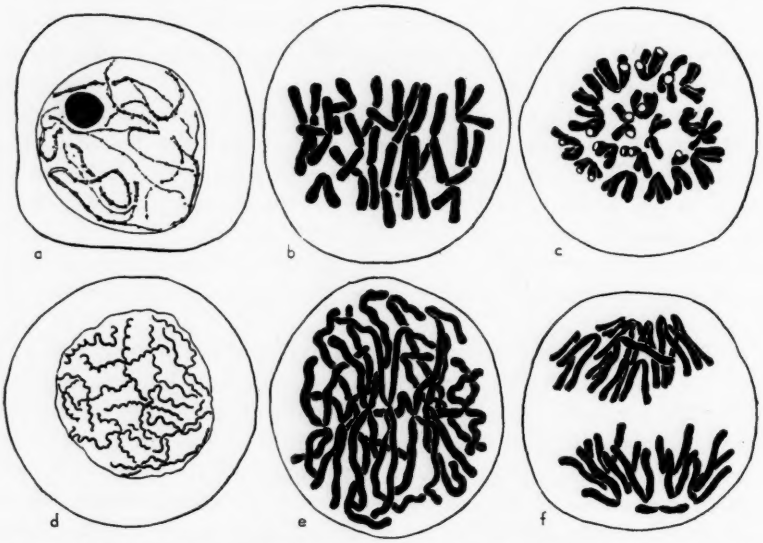


Fig. 6. Meiosis of the asynaptic type, the general outlines, *a*: pachytene, *b*: metaphase I, side view, *c*: a somewhat later stage of metaphase I, polar view, *d*: interkinesis, *e*: metaphase II, *f*: anaphase II. — × 1400.

were overtaken by the telophase, while the centrosomes were still keeping them strictly arranged on the equatorial plate.

Thus a uninuclear interkinesis stage is formed with complete regularity (Fig. 6 *d*), a kind of restitution nucleus containing the total of the first meiotic chromosome quantity. Even in these cases, where from the beginning numerous micronuclei were formed, they later on fuse into one single nucleus. And if in exceptional cases more than one interkinesis nucleus are maintained until the beginning of the homeotypic division, they will never form separate spindles in this division, they will instead be included in the main spindle.

During the interkinesis stage a clear spiral structure of the chromosomes may be seen (Fig. 5 *a*—*c*). When the nuclear membrane disappears at the onset of the second division, the centromeres are already from the beginning arranged in the equatorial plane, while the chromosome arms are folded towards the poles (Fig. 5 *d*—*i*). The chromosomes now fill up the cell in quite a different manner from that at the first metaphase (Fig. 6 *e*).

Now the centromeres are finally divided and a normal anaphase starts (Fig. 6 *f*). This proceeds normally and the result is pollen dyads with somatic chromosome number. The regularity of this meiosis type is proved very clearly by the conditions of the pollen described in detail in the next chapter. All investigated pollen grains have exactly 21 chromosomes. Thus, the asynaptic meiosis is a rather unique way in which a triploid species has succeeded in acquiring the ability of forming pollen grains with balanced chromosome conditions.

5. THE MITOSIS OF THE POLLEN GRAIN.

a. *The normal type.* — The pollen tetrads of the synaptic forms have the normal *Allium* appearance. The pollen grains are crescent in shape (Fig. 7 *a*, Fig. 8 *a*). As expected, 14 is the most common chromosome number of the pollen, but the number 13 is also found fairly often. In a couple of slides the following frequency of chromosome numbers was noted:

Number of chromosomes:	12	13	14	15	Total	Med.
Number of cases:	2	15	24	3	44	13,6

b. *The asynaptic type.* — The asynaptic dyad pollen is semispherical in shape (Fig. 8 *b*) and resembles completely the pollen grains originated by monokinetic meiosis (LEVAN, 1935 b, Fig. 46). The first mitosis of the asynaptic pollen grains occurs quite regularly. The chromosome number is 21 (Fig. 7 *b*). The spindle is asymmetric and at anaphase the larger anaphase group goes up distally into the pollen cupola, while the more condensed group is pressed towards the proximal, plane pollen wall. The former group forms the vegetative nucleus and the latter the generative nucleus. The asynaptic pollen shows the same germinating power on agar as normal pollen. The

division of the generative nucleus occurs in the pollen tube about 24 hours after the onset of germination.

The constancy in chromosome number of the asynaptic pollen is in fact rather remarkable. All 36 metaphase plates of one slide, the chromosome number of which could be determined with absolute certainty, contained exactly 21 chromosomes. This furnishes better than anything else a picture of the regularity with which the asynaptic meiosis takes place. In this respect *Allium amplectens* is different from previously described cases of asynapsis, where the simultaneous presence of univalents and bivalents upsets the balance of meiosis.

Among the asynaptic dyad pollen there is always a very low but

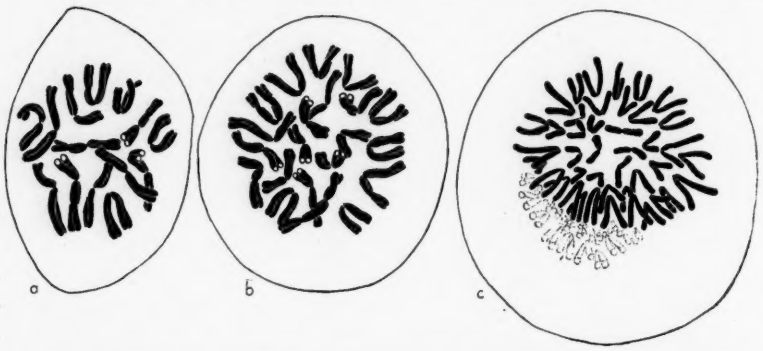


Fig. 7. The first pollen mitosis, *a*: normal tetrad pollen (n = 14), *b*: asynaptic dyad pollen (n = 21), *c*: asynaptic monad pollen (n = 42), *a*, *b*: metaphase, *c*: anaphase. — × 1200.

regular percentage of monad pollen. An idea of its frequency may be had from the fact that in one slide (= 5/2 anthers) 30 monads were present. The frequency is probably less than 1 ‰. The mode of origin of the monad pollen grains could not be directly observed, owing to their extreme rarity. I think, however, that there is little doubt that they originate by monokinetic meiosis, which is a very common process in *Allium*. In this case, where also the first division is omitted, it should perhaps more appropriately be called akinetic meiosis.

The monads are spherical in shape and their diameter is decidedly larger than in the dyad pollen. One monad pollen grain can be seen in the microphoto, Fig. 8 *b*, below the centre at about 7 o'clock. The increase in cell diameter is of interest. Evidently the plane, proximal pollen wall of the dyads puts a limit to their further growth in size, whereas the spherical monads may continue growing still further.

The chromosome number of the monads has been directly counted only in a few cases. In a couple of anaphases, one of which is pictured in Fig. 7 *c*, there were 42 chromosomes present, i. e. the double somatic number. Although those moments of asymmetry, due to the tetrad division, are absent in the monads, their pollen mitosis is markedly asymmetric. One anaphase group is spread out in the centre of the cell, developing into the vegetative nucleus, the other group is pressed against the wall into the flattened, lentiform, generative nucleus. The situation

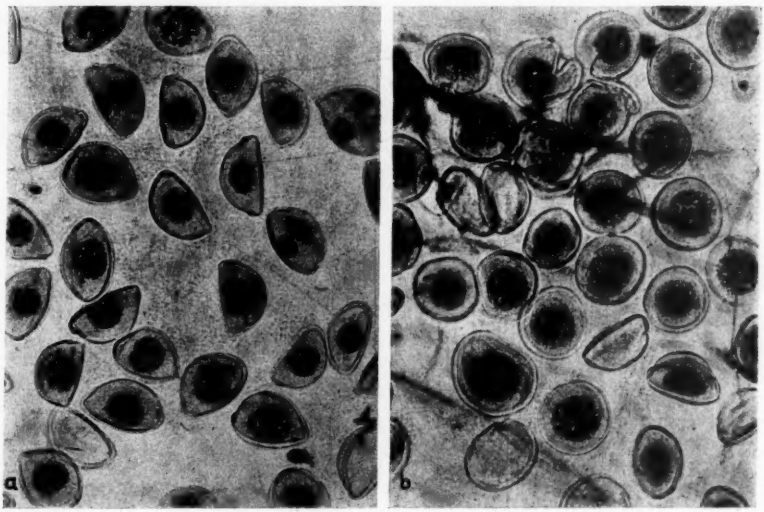


Fig. 8. Microphoto of pollen grains of *a*: normal type, *b*: asynaptic type. — × 300. Microphoto OTTO MATTSSON.

is exactly similar to that seen in the artificially produced monad pollen (LEVAN, 1939). The monad pollen, too, may be easily germinated on agar.

It should be observed that the 21-chromosome pollen grains of the asynaptic form all contain the same genic quantity as somatic cells. No genotypic variation occurs between different pollen grains, a variation generally present elsewhere among the gametes of triploid forms. It is therefore possible here to make a comparison between the purely modificative chromosome variation between different pollen grains and the variation due to genetic segregation. The genic variation is especially pronounced in plants which are hybrids between

types with different chromosome size. This condition is seen very plainly in the hybrid *Allium Cepa* × *fistulosum*. Other instances of this are recorded by UPCOTT (1939). She emphasizes that the difference in size is due to a varying spiralisation »owing to the direct action of the individual genotype». It must be said, however, that even in the pollen of the asynaptic *Allium amplectens* there occurred a greater variation in chromosome contraction between different pollen grains than is generally seen in the root tissue of *Allium*. No doubt, however, the variation was somewhat less than in the pollen of synaptic triploids and hybrids.

6. THE NUCLEOLI.

Allium amplectens is, compared with most other *Allium* species, characterized by deviating nucleolar conditions. In most *Allium* species

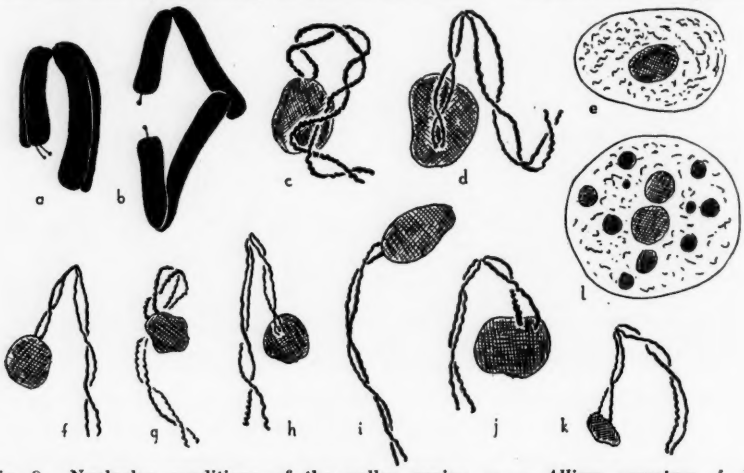


Fig. 9. Nucleolar conditions of the pollen grains, *a—e*: *Allium serratum*, *f—l*: *Allium amplectens*, *a*: the s_1 chromosome at metaphase, *b*: at anaphase, *c—d*: 2 instances of the nucleolar chromosome, *e*: vegetative resting nucleus, *f—k*: 6 nucleolar chromosomes from one nucleus, *l*: vegetative resting nucleus. — *a—d*, *f—k*: × 3250. *e*, *l*: × 1600.

there is present one satellited chromosome (the »s_1») in each genome, and the nucleolus is formed at the achromatic portion of this s_1. Thus diploid species usually develop two nucleoli in somatic cells and one nucleolus in the pollen.

As mentioned above, no s_1 could be identified in *Allium amplectens*, and its nucleolar conditions are irregular. In recently divided somatic cells a great many nucleoli are formed. Systematic counts of the

nucleoli could not be made, since the conditions in somatic tissues are rather irregular and not so clear unless special stainings are used, but 10 or 12 nucleoli in each cell are very often present. If the number of nucleoli is great, they are tiny in size, if one or two decidedly larger nucleoli are present, the number of nucleoli is less. Thus it is clear that the nucleoli fuse.

In the archesporium and in very young pollen mother cells there are many nucleoli (about 4—10). But in the prophase stages of meiosis, pachytene and diplotene, not more than one nucleolus is seen. It is not very large and cannot be the result of the fusion of many nucleoli. It seems to be attached to the end of one paired pachytene chromosome.

The nucleoli are more clearly observable during the pollen development. In the prophase of the first pollen mitosis several nucleoli per cell are present. In the different types the following numbers were counted:

Number of nucleoli:		1	2	3	4	5	6	7	Total	Med.
Number of cases	Synaptic type: ...	1	11	18	4	—	—	—	34	2,7
	Asynaptic type: ...	—	1	1	16	9	6	2	35	4,7

At late prophase it may be seen that the nucleoli are attached to individual chromosomes. In Fig. 9 *f*—*k* 6 such prophase chromosomes from the same cell of the asynaptic type are reproduced.

At late telophase of the pollen mitosis the nucleoli are present in great numbers. The following number was counted on one occasion:

Number of nucleoli:		3	4	5	6	7	8	9	10	Total	Med.	Med. Genome
Number of cases	Synaptic type: ...	5	20	8	3	—	—	—	—	36	4,3	2,1
	Asynaptic type:...	—	—	1	5	12	9	4	2	33	7,2	2,4

Thus it may be concluded that the development of the nuceloli in *Allium amplectens* is not associated with one determined chromosome of the genome. 6 chromosomes of the synaptic pollen and 10 or even more of the asynaptic pollen are capable of producing nucleoli. Calculated on the number of chromosomes present between 2 and 3 chromosomes per basic genome may function as nucleolus carriers.

As a comparison I shall briefly mention the nucleolar conditions of the pollen of the diploid Californian species *Allium serratum*. Its basic chromosome set is of the same morphological appearance as the *amplectens* genome apart from the important difference that *Allium serratum* has a typical s_1 chromosome (Fig. 9 *a*, *b*). In the prophases

Fig. 10. Inflorescence of *Allium amplectens* showing the crests of the seed capsules.

and resting nuclei of the pollen there occurs regularly only one nucleolus (Fig. 9 *e*). This is formed distally on one chromosome, probably the s_1 (Fig. 9 *c*—*d*). I have not, however, been able to recognize the satellite in this stage, but its presence has been ascertained in several other *Allium* species.

Between *Allium amplectens* and *serratum* there occurs a similar distinction to that MATSUURA (1938) observed between *Trillium* and

Paris. In the former, satellites are lacking and the nucleoli may be formed on several chromosomes, in the latter case a typical satellited chromosome is present and functions as the satellite organizer. In agreement with these findings MATSUURA divides the nucleolar chromosomes into two main types: *1.* the ordinary interstitial type, where the nucleolus is formed at the achromatic portion of the satellited chromosomes, and 2. the terminal type, where nucleoli may be formed at the end of several chromosomes. In this latter case certain chromosomes often prevail as nucleolar organizers and there is a kind of competition between the different organizers. If for some reason or other the satellite-carrying portion of the interstitial type is lost, a transition to the terminal type may be brought about. Instances of this were found by MCCLINTOCK (1934) in *Zea*. Pollen grains lacking the nucleolus organizer of the chromosome 6 developed many small nucleoli. In other cases micronuclei devoid of the satellited chromosome have been seen to develop nucleoli.

The »Ubiquität» of »SAT» chromosomes, argued by RESENDE (1937), must, it is true, be considered to be valid in most cases. Certain exceptions to the rule cannot, however, in my opinion, be denied. It seems impossible to assume that in *Allium amplectens* so many satellited chromosomes should have been overlooked as is indicated by the number of nucleoli. Especially in the pollen mitoses the chromosomes are so clear that even very small satellites should have been observed in some of the hundreds of metaphase plates which have been analysed. Thus it is very tempting to assume, in accordance with MATSUURA, that the difference in nucleolar conditions between *Allium amplectens* and neighbouring species is due to the lacking of satellited chromosomes in *Allium amplectens*.

II. HERBARIUM STUDIES.

1. HISTORICAL SKETCH.

In order to study the occurrence in nature of the asynaptic meiosis of *Allium amplectens* it was found necessary first to make a brief examination of the taxonomy of the species and to determine the geographical distribution of the species and of its main types. In the present chapter a short survey will be given of the taxonomical characters of the species as they have been described by different authors.

The species *Allium amplectens* was described for the first time by

TORREY (1856) on material from Sonoma County, California. The name *amplectens* denotes the condition of the spathal leaves: »spatha e bracteis 2 orbiculatis concavis subacuminatis flores amplectentibus». This character is especially evident in the few-flowered forms. TORREY also concludes his description as follows: »Easily distinguished by the small few-flowered umbel, which is almost enclosed in the concave purple bracts».

KELLOGG (1861) described another type under the name of *Allium attenuifolium*, which according to current opinion belongs to *amplectens*. Describing material from Mt. Shasta (Siskiyou County, California), he emphazises the leaf characters of this form: »Leaves two, radical, stem sheathing at the broad membranous base, striate and channelled below, closely canaliculate above, very narrow and slenderly attenuated toward the filiform apex; margins slightly scabrous». KELLOGG's type is a stout many-flowered plant: »Umbel globose, many-flowered (50 to 80 or more)». He detects the important character of crests on the wall of the seed capsule: »Germ, color lively, pinkish capsule, turbinate, sub-three angled or three rounded cells, each cell slightly or obsoletely two-crested, central axis at the pistil depressed».

Allium amplectens afterwards reappears in GREY (1867) as *Allium occidentale*, while WOOD (1868) partly retains the name *amplectens* with a verbatim quotation of TORREY's diagnosis, partly takes up another name, *acuminatum* HOOK. *β gracile*, for *amplectens* material from Butte County, California. REGEL (1875) who generally treats the American species somewhat summarily, places *amplectens* as a synonym of *serratum* WATS.

GREENE (1894), in his »Manual of the Botany of the Region of San Francisco Bay», mentions KELLOGG's *Allium attenuifolium* and gives the important description of the structure of the bulb scales: »Bulb-coats white, with a delicate transversely sinuate or serrate reticulation, the vertical lines especially also minutely sinous». This character is easily recognizable and distinguishes the species from related species. JEPSON, in connection with *Allium attenuifolium* in GREENE's Flora, describes a new species, *Allium monospermum*, which is characterised by »capsule (by abortion) 1-celled, 1-seeded». The type-locality is Vaca Mts. in Solano County, California. In his own »Flora of California», however, JEPSON (1922) combines these two species under *Allium amplectens* TORR. Other modern Floras, for instance, ABRAMS (1923), JEPSON (1925) and MUNZ (1935), agree rather well as to the limitations of *Allium amplectens*.

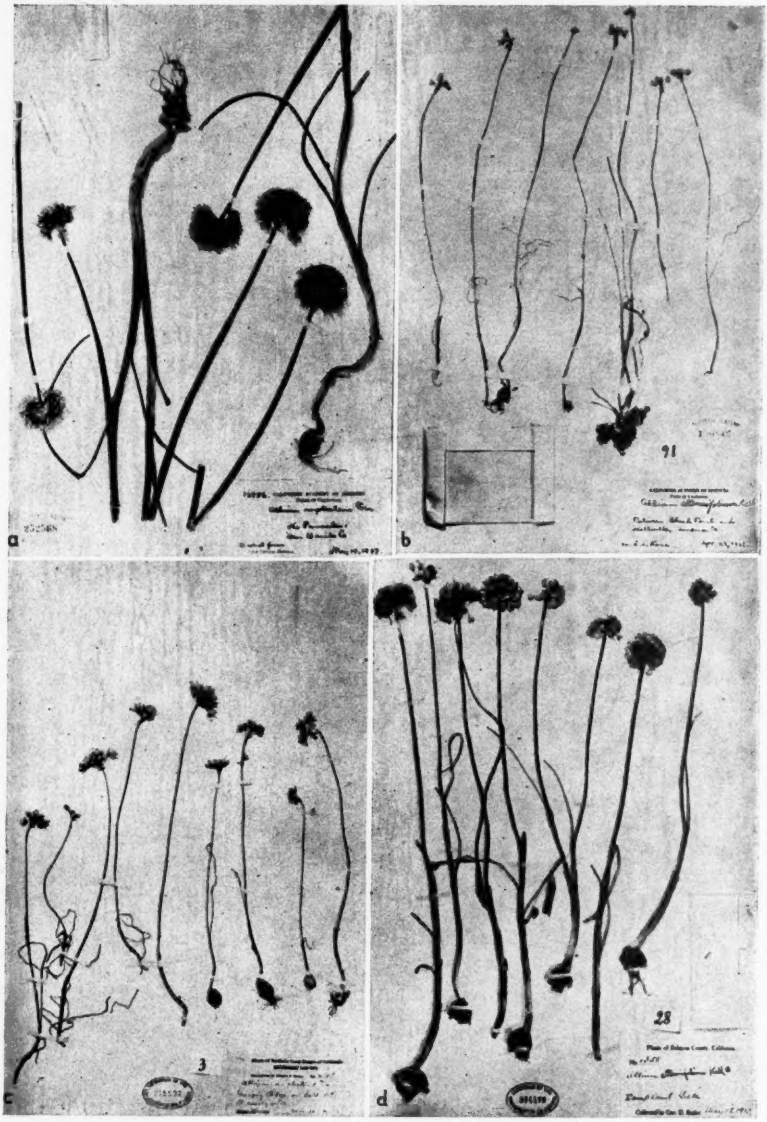


Fig. 11. Different types of *Allium amplectens*, *a*: San Benito Co., *b*: Sonoma Co., *c*: Humboldt Co., *d*: Siskiyou Co.

From the above facts will be seen that *Allium amplectens* is a rather multiform species. As the different collections began more completely to represent the different regions of its distribution, it became gradually possible to obtain a clearer insight as to what characters were of taxonomic value and which varied from form to form. This will be seen very clearly from the following list, comprising some of the taxonomic authors' descriptions of the different organs of *Allium amplectens*.

Bulb. — TORREY: large for the size of the plant; KELLOGG: (about *attenuifolium*) small, roundish, truncated; GREENE: bulb-coats white; JEPSON: bulb-coats commonly reddish; MUNZ: bulb-coats reddish to greyish.

Scape. — TORREY: scapo flexuoso spithamaeo superne bifoliato; KELLOGG: scape terete, solid, glaucous, smooth (minutely speckled); GREENE: scape 10—18 in. leafy below.

Leaves. — TORREY: foliis filiformibus, leaves scarcely a line wide, overtopping the scape; KELLOGG: leaves two; GREENE: leaves several, very long and slender; ABRAMS: leaves 2—4, shorter than the scape, narrow becoming convolute-filiform above the sheathing base.

Spathe. — TORREY: e bracteis 2; KELLOGG: bracts 3, outermost larger, broad-ovate or oblong-ovate, short acuminate, sessile, membranous, 4- to 9-nerved or more; GREENE: bracts 2, short, abruptly pointed; JEPSON: bracts 3; ABRAMS and MUNZ: bracts 2.

Umbel. — TORREY: pauci (3—6) flora; KELLOGG: many-flowered (50—80 or more); JEPSON: (about *monospermum*) pedicels 50—80 (in his floras 1922 and 1925) pedicels 25—35, umbel erect, usually dense; ABRAMS: umbel dense, almost capitate; MUNZ; pedicels 10—40, slender, 5—12 mm long.

Flower colour. — KELLOGG: whitish (scarcely a pinkish tinge?), midrib of the petals pinkish; GREENE: white; JEPSON: (*monospermum*) pale-purplish, (1922, 1925) white or nearly so; ABRAMS: white or tinged with pink.

Perianth. — TORREY: sepalis oblongis obtusiusculis; KELLOGG: petals ovoid-diamond-acute, slightly inflexed from the middle, the three inner a little narrower; GREENE: oblong-lanceolate-acuminate segments, 3—4 lines long; MUNZ: narrowly oblong-ovate, 6—8 mm long.

Stamens. — TORREY: filamentis e basi lata submonadelpha subulatis; KELLOGG: filaments inserted at the base, subulate, white; JEPSON: (*monospermum*) filaments with broadly deltoid and connate bases.

KELLOGG: stamens as long as the perianth; JEPSON: perianth more

or less exceeding the stamens and style; ABRAMS: stamens scarcely shorter than the perianth; MUNZ: stamens shorter than the perianth.

Capsule. — TORREY: capsula trigastrica, apice depressa, loculis dispermis; KELLOGG: cells 2-seeded, rarely more than one perfected; WOOD: semine unico; JEPSON: (*monospermum*) capsule 1-celled, 1-seeded.

From the often discrepant data cited above a rather good picture may be had of the variation of the species. In the subsequent list of herbarium specimens this picture will perhaps be supplemented in a few respects.

2. HERBARIUM MATERIAL EXAMINED.

In the following list of herbarium specimens some morphological observations are given, especially if the types examined deviate in some way or other from the normal *amplectens* type, or if several specimens from the same region or similar type of locality show a decided agreement in general appearance, which has been interpreted as characteristic of a certain ecotype. The specimens are arranged geographically into different counties; see the map (Fig. 12). For each listed specimen the following data are given: locality, my cytological number (in *italics*), year of collection, collector, herbarium in which the specimen is kept (B = University of California, Berkeley; C = California Academy of Science, San Francisco; D = Dudley Herbarium, Stanford University).

CALIFORNIA.

1. San Diego Co. Cuyamaca, *35*, 1880, S. B. and W. F. PARISH, D; *36*, 1903, L. R. ABRAMS, D; *21*, *37*, 1928, I. L. WIGGINS, BD; *5*, 1932, M. E. JONES, B.

This, the most southern locality of the species, is isolated from the rest of its distribution area. All the studied specimens are of the same, very characteristic type: scapes erect, rather thick and stiff, umbels dense, capitate, pedicels short. All specimens have asynaptic pollen.

2. San Luis Obispo Co. San Simeon, *129*, 1936, L. S. ROSE, C.

3. Monterey Co. Jolon, *127*, 1935, D. D. KECK and P. STOCKWELL, C.

These two specimens from the southern coastal range are of a similar habit. Both are low and have somewhat arched, slender scapes. The umbels are thin, pedicels of different length. Pollen normal, *129* is diploid, *127* tetraploid, although *127* is more slender and gracile than *129*, and has fewer flowers per umbel.

4. Tulare Co. Visalia, *17*, 1898, P. S. WOOLSEY, B; 3 Rivers, *75*, 1925, L. R. ABRAMS, D; Middle Tule River, PURPUS (JEPSON, 1922).

17 and *75* deviate very much from other types of *Allium amplectens* with their long pedicels and large star-shaped flowers. They possibly belong to another species. Pollen normal, both diploid.

5. San Benito Co. Hernandez, *68*, 1903, L. M. LATHROP, D; Pinnacles, *124*, 1937, J. T. HOWELL, C.

68 is a small, delicate type with upright scapes. Pollen normal, tetraploid. *124* is tall (5 dm) and robust, thick scapes, large spherical umbels (Fig. 11 *a*). Pollen asynaptic.

6. Mariposa Co. Mariposa, *66*, 1889, *70*, 1892, *19*, 1893, 1894, *13*, no date, J. W. CONGDON, BD; *84*, 1914, S. FAUNTLEROY, C; Mt. Bullion, *7 b*, no date, S. SUNEY, B.

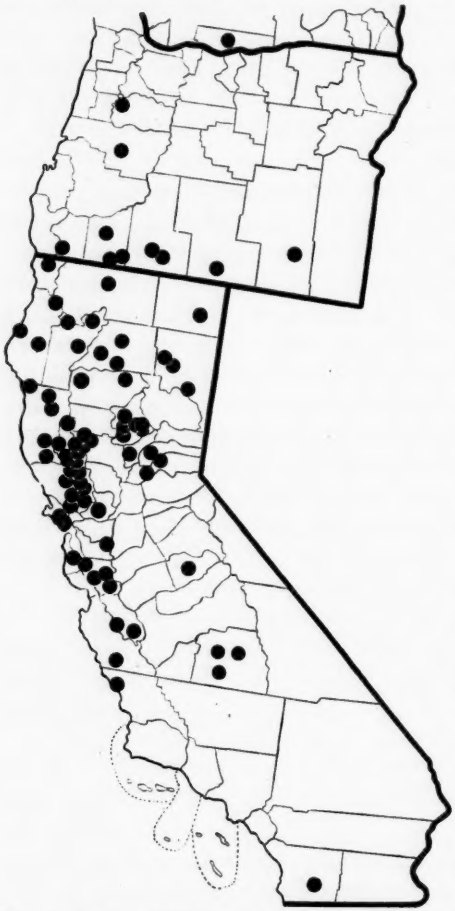


Fig. 12. The distribution of *Allium amplectens*.

These are similar in type, erect scapes, umbels small and dense. Both normal and asynaptic pollen, types with normal pollen are tetraploid.

7. Santa Clara Co. Coyote Cr., *62*, *63*, 1895, W. R. DUDLEY, D; Stanford University, *65*, 1898, L. R. ABRAMS, D, *4*, *59*, *89*, 1903, A. D. E. ELMER, BCD; Uvas-Almaden Rd. *64*, 1922, R. S. FERRIS, D; Gilroy Hot Spr., *111*, 1937, A. EASTWOOD and J. T. HOWELL, C; Isabel Cr., *133*, *134*, *135*, J. CLAUSEN, D. D. KECK and A. LEVAN.

8. San Mateo Co. Jasper Ridge, *61*, 1921, H. L. MASON, D; Stanford University, *58*, 1932, L. R. ABRAMS, D.

The specimens from Santa Clara and San Mateo are rather consistent in type, medium-sized, normal. With the exception of some of the forms from Isabel Cr. all have normal pollen and are tetraploid (*64*, however, which deviates considerably from *amplectens*, is a diploid).

9. Alameda Co. Cedar Mt., *60*, *88*, *98*, 1903, A. D. E. ELMER, CD.

10. Contra Costa Co. Mt. Diablo, *97*, 1902, W. W. CARRUTH, C.

Simple erect scapes with leaves only in their lowest part. They all agree in habit. From Cedar Mt. the diploid *88* and the tetraploid *60* should be compared. The diploid is evidently larger and more robust than the tetraploid. *98* has mixed pollen, normal and asynaptic, even within one stamen, the others have normal pollen.

11. Marin Co. Mt. Tamalpais, *30*, 1892, T. S. BRANDEGEE, B; Lagunitas, *15*, 1893, W. C. BLESDALE, B; Kentfield, *100*, 1912, M. E. PARSONS, C.

30 and *100* very stout types with big bulbs, *15* more slender. All three have normal pollen, tetraploids.

12. Sonoma Co. Hill-sides, Sonoma, type locality of the species (TORREY, 1856); Hood's Peak, *12*, 1892, MICHENER and BIOLETTI, B, *93*, *110 b*, *130*, 1927, M. S. BAKER, C; Petrified Forest, *85*, 1915, A. EASTWOOD, C; Adobe Canon, *123*, 1927, M. S. BAKER, C; Shellville, *91*, 1931, L. S. ROSE, C.

91, *93*, *110 b* and *130* are of a very characteristic type. Groups of small rounded bulbs grow together »in bunches of 6—30», evidently in moist places. The scapes are very slender and gracile, often somewhat undulated, the inflorescences are small, few-flowered, pedicels short. These four specimens have normal pollen and are diploid. This form will be referred to later as the Sonoma-type (Fig. 11 *b*). *12* and *85* are of a more common, taller and stouter growth. Pollen normal, tetraploids.

13. Napa Co. Howell Mt., *18*, 1899, J. P. TRACY, B; Walter's Spr., *55*, 1933, D. D. KECK, D; Napa, *120*, *121*, 1933, J. T. HOWELL, C; Palisades, *112*, 1937, M. S. JUSSEL, C.

120 agrees completely with the Sonoma-type: »growing in dense clumps from numerous small bulbs». Similar to the earlier specimens of the Sonoma-type, it has normal pollen but is evidently a tetraploid. *121* was collected in the same region, had »stems growing singly» and was of quite another general appearance: big, marked bulbs, stout, short scapes, large flowers. It has asynaptic pollen. *18* reminds of *120* but is lower and more slender, normal pollen, diploid. *55* and *112* are both tetraploids, and they are of strong and robust growth. Normal pollen.

14. Solano Co. Vaca Mt. (JEPSON, 1922).

15. Lake Co. Lakeport, *16*, 1902, J. P. TRACY, B; Lower Lake, *53*, 1902, A. M. BOWMAN, D, *86*, 1932, M. S. JUSSEL, C, *79*, *80*, 1938, A. EASTWOOD and J. T. HOWELL, C; Mt. St. Helena, *105*, *106*, 1915, A. EASTWOOD, C; Cache Cr., *54*, *103*, 1919, A. A. HELLER, CD, *56*, 1928, D. K. KILDALE, D; Kelseyville, *90*, 1924, J. B. BLANKINSHIP, C; Adams Spr., *114*, *116*, 1933, M. S. JUSSEL, C; Oak Park Spr., *6*, *57*, 1933, R. BACIGALUPI, R. S. FERRIS and I. L. WIGGINS, BD; Lake-Colusa Boundary, *78*, 1934, C. PURDY, C; Indian Valley, n. e. Lake Co. (JEPSON, 1922).

Most of these specimens represent stout, erect types. *90* and *114* are more gracile. 11 of them are tetraploids with normal pollen, 4 are asynaptic, only 1 is diploid with normal pollen.

16. Colusa Co. Stonyford, *74*, 1926, R. S. FERRIS, D; Williams, *117*, 1934, K. ESAU, C.

Both asynaptic.

17. Mendocino Co. Red Mts., *22*, 1901, A. EASTWOOD, B; *113*, 1937, A. EASTWOOD and J. T. HOWELL, C; Covelo, *52*, 1903, V. RATTAN, D, *87*, 1928, A. EASTWOOD, C; Ukiah, *108*, 1913, A. EASTWOOD, C; Hopland, *7*, 1921, J. P. TRACY, B, *115*, *122*, 1936, A. EASTWOOD and J. T. HOWELL, C; Hearst, *50*, 1927, R. BACIGALUPI, D.

All agree rather well in type, *115* and *122* have unusually rich-flowering inflorescences. 6 of them have normal pollen and are tetraploids, while 3 are asynaptic.

18. Humboldt Co. Kneeland Prairie, *51*, 1903, V. RATTAN, D, *30 b*, 1912, *1*, 1921, J. P. TRACY, B; Bald Mt., *3*, 1923, J. P. TRACY, B; Yager, *42*, 1923, J. P. TRACY, D, *131*, 1937, A. EASTWOOD and J. T. HOWELL, C; Van Duzen River, *126*, 1936, C. C. and S. K. HARRIS, C (Plantae exsiccatae Grayanae, No. 645).

3 is a very gracile diploid with normal pollen (Fig. 11 *c*), *126*, tall, rich-flowered, asynaptic, the others are stout and tall tetraploids with normal pollen.

19. Del Norte Co. Gasquet, *32*, 1935, H. E. PARKS and J. P. TRACY, B.

Tall, rather slender form with normal pollen, tetraploid.

20. *Placer Co.* Auburn, *92*, 1915, E. HANVER, C; Colfax, *110*, 1932, B. R. JACKSON, C.

21. Nevada Co. Nevada City, *101*, 1912, A. EASTWOOD, C; Banner Hill, *23*, 1916, H. M. HALL, B; Grass Valley, *99*, 1919, A. A. HELLER, C.

All these specimens from Placer and Nevada show a great similarity in general appearance: small rounded bulbs, erect, gracile scapes, small umbels; they have all normal pollen and all, except *92*, are diploid. The tetraploid form *92*, however, does not deviate from the others morphologically.

22. Yuba Co. Marysville, *95*, 1930, A. EASTWOOD, C.

Differs very much from the preceding specimens: tall, stout, rich-flowering. Pollen normal, tetraploid.

23. Butte Co. Grain Fields, *24*, 1896, R. M. AUSTIN, B; Clear Cr., *72*, 1897, H. E. BROWN, D, *69*, *70 b*, 1902, A. A. HELLER and H. E. BROWN, D; Chico, *9*, 1898, BRUCE, B, *8*, *73*, *102*, 1914, A. A. HELLER, BCD; Oroville, *94*, 1931, L. S. ROSE, C; Durham, *81*, 1935, F. BROWN, C; Paradise, *119*, 1936, M. E. WALL, C.

Among these an erect, rather slender type predominates. Its umbels are small, dense, capitate. *119* approaches the tufted Sonoma-type. *9* and *94* are more robust. All have normal pollen and all, except the gracile type *119*, are tetraploids.

24. Tehama Co. Paynes Cr., *76*, 1930, D. K. GILLESPIE, D, *49*, *118*, 1934, A. EASTWOOD and J. T. HOWELL, DC; Rosewood (JEPSON, 1922).

Normal pollen, tetraploids.

25. Shasta Co. Anderson, *83*, *109*, 1913, L. E. SMITH, C; Kennett, *107*, 1913, L. E. SMITH, C; Montgomery Cr., *104*, 1923, E. BETHAL, C; Redding, *82*, 1934, A. EASTWOOD and J. T. HOWELL, C.

82 and *107* tall, the others small and tender, especially 104, which is very gracile with small umbels and short pedicels. They have all normal pollen and are tetraploids.

26. Trinity Co. Union Cr., 25, 1909, H. M. HALL, B; Scott Mts., *128*, 1931, A. EASTWOOD and J. T. HOWELL, C; Eagle and Bear Cr. *125*, 1937, A. EASTWOOD and J. T. HOWELL, C.

25 and *128*, low, gracile, few-flowered, resemble Shasta *104*. They have asynaptic pollen. *125* decidedly more vigorous, normal pollen, diploid.

27. Siskiyou Co. Yreka, *28*, *71*, 1910, G. D. BUTLER, BD; Mt. Shasta, A. A. VEATCH (type locality for *Allium attenuifolium*; KELLOGG, 1861).

28 (Fig. 11 *d*) and *76*, robust, erect, both asynaptic.

28. Lassen Co. Susanville, *10*, 1892, T. S. BRANDEGEE, B; Dixey Mts. *11*, *31*, 1894, M. S. BAKER and F. NUTTING, B; Pine Cr., *14*, 1898, C. C. BRUCE, B.

11 and *14*, strong, robust, very large umbels, *10* and *31*, low, tender, small umbels, very short pedicels. All except *10* are asynaptic. The very striking difference in size between *11* and *31*, which were collected on the same occasion, together with the difference in pollen size makes the conclusion drawn in another connexion highly probable, that different chromosome numbers may occur among asynaptic forms as well as among types with normal pollen.

29. Modoc Co. 31 c, 1893, M. S. BAKER, B; Altura, *67*, 1919, R. S. FERRIS and R. DUTHIE, D.

67 resembles the small Lassen types, asynaptic.

OREGON.

30. Josephine Co. Waldo, *77*, 1928, Kerby, *77 b*, 1928, J. W. THOMPSON, D.

77 very small and delicate, leaves filiform. Normal pollen, tetraploid.

31. Jackson Co. Rogue River, *29*, 1893, R. M. AUSTIN, B; Siskiyou Mts., *44*, 1894, F. M. ANDERSON, D; Chinquapin Mt., *47*, 1925, E. J. APPELGATE, D; Pinehurst, *38*, *40*, 1927, M. E. PECK, D.

38, *40* and *47* have normal pollen, tetraploids, the others have asynaptic pollen.

32. Klamath Co. Swan Lake, *48*, 1923, E. J. APPELGATE, D; Bonanza, *39*, *43*, 1927, M. E. PECK, D.

43 has normal pollen, tetraploid, the others asynaptic.

33. Lake Co. Lakeview, *46*, 1927, M. E. PECK. D.

Tall, stout, small inflorescences, asynaptic.

34. Harney Co. Steen Mt. *34*, 1896, J. B. LEIBERG, B, *33*, 1898, W. C. CUSICK, B, *45*, 1925, M. E. PECK, D.

Asynaptic.

35. Lane Co. Dorena, *2*, 1924, L. CONSTANCE, B.

Small, tender, filiform leaves, normal pollen, diploid.

36. Linn Co. Albany, *41*, 1928, J. W. THOMPSON, D.

Tall and stout, normal pollen, tetraploid.

WASHINGTON.

37. Klickitat Co. According to PIPER (1906), *Allium attenuifolium* occurs in a collection, SUKSDORF 60, from this county. In reply to an inquiry on this matter, Dr. C. W. SHARSMITH, Pullman, very kindly gave me the following information in a letter: »After a careful search not only through the herbarium, but also through PIPER's and SUKSDORF's as yet unmounted material, I failed to find the SUKSDORF 60 specimen cited by PIPER. However, I did find among SUKSDORF's unmounted plants a single sheet containing rather scanty, albeit sufficient material for verification, of *Allium amplectens* TORR. This SUKSDORF collection was unnumbered; it is named by him »*A. attenuifolia*» and is given the locality in his own handwriting as »WS 24 May, 1881». The »WS» I interpret as White Salmon (in western Klickitat County, Washington), on the basis of our knowledge as to his whereabouts at this time . . .». From this it is clear that *Allium amplectens* goes as far north as to the southern parts of Washington.

3. PLANT-GEOGRAPHICAL SURVEY.

By putting together the data from the different specimens of *Allium amplectens*, listed in the preceding chapter, material is furnished of a rather detailed distribution map of the species. 120 of the specimens are from California and 16 from Oregon. To this may be added some

localities from the literature. If different specimens, which might possibly represent the same locality, are combined, the following result is reached: 68 certainly different localities in California, 10 in Oregon and 1 in Washington. These latter are the data plotted on the map (Fig. 12).

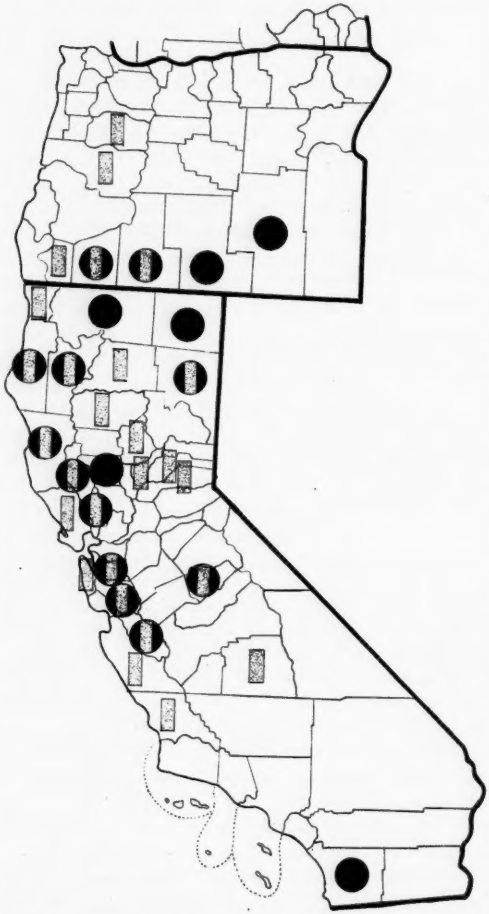


Fig. 13. The distribution in nature of normal and asynaptic meiosis in *Allium amplectens*, rectangular dots = normal meiosis, round dots = asynaptic meiosis, rectangle within round dots = both types occur.

It may be seen from this map that the distribution of the species extends along the Pacific Coast from southern California into southern Washington. Its main occurrence is northern California, and its densest distribution is found in Napa and Lake Counties in California. Possibly, however, this density is only apparent, since this region may be more frequently visited by botanists. Thus Dr. H. L. MASON informs me in a letter: »Certain counties of the Sierra Nevadas have not been intensively collected, and it is extremely likely that the species occurs in all of the counties north of Tulare».

Pollen samples were examined from so many herbarium specimens as possible. Acetocarmine slides turned out to be very useful. It was possible in each case to decide with certainty if

TABLE 1. *The occurrence of asynapsis in the different counties.*

Normal pollen						Asynaptic pollen			Both types of pollen							
County No.	County	Number of specimens	County No.	County	Number of specimens	County No.	County	Number of specimens	County No.	County	Number of specimens		County No.	County	Number of specimens	
											Normal pollen	Asynaptic pollen			Normal pollen	Asynaptic pollen
2	San Luis Obispo	1	21	Nevada ...	3	1	San Diego.	5	5	San Benito	1	1	28	Lassen.....	1	3
3	Monterey	1	22	Yuba	1	16	Colusa	2	6	Mariposa..	2	2	31	Jackson ...	2	2
4	Tulare	2	23	Butte	11	27	Siskiyou ...	2	7	Santa Clara	9	1	32	Klamath ...	1	2
8	San Mateo	1	24	Tehama ...	3	29	Modoc	1	9	Alameda ...	2	1				
10	Contra Costa ...	1	25	Shasta	5	33	Lake Oreg.	1	13	Napa	4	1				
11	Marin	3	30	Josephine .	1	34	Harney ...	3	15	Lake Calif.	12	4				
12	Sonoma	5	35	Lane.....	1				17	Mendocino	6	3				
19	Del Norte	1	36	Linn.....	1				18	Humboldt.	5	1				
20	Placer	2							26	Trinity ...	1	2				
				Total	43		Total	14						Total	46	23

normal or asynaptic pollen was present. Among the 126 specimens, where this examination could be carried out, no less than about $^1/_3$ (37 specimens) showed asynaptic pollen, while 89 specimens had normal pollen. When these conditions were plotted on the map it was found that the phenomenon of asynapsis had a very wide occurrence in nature: Among 34 counties represented, 16 had only normal pollen formation, 5 had purely asynaptic pollen and 12 had both types of

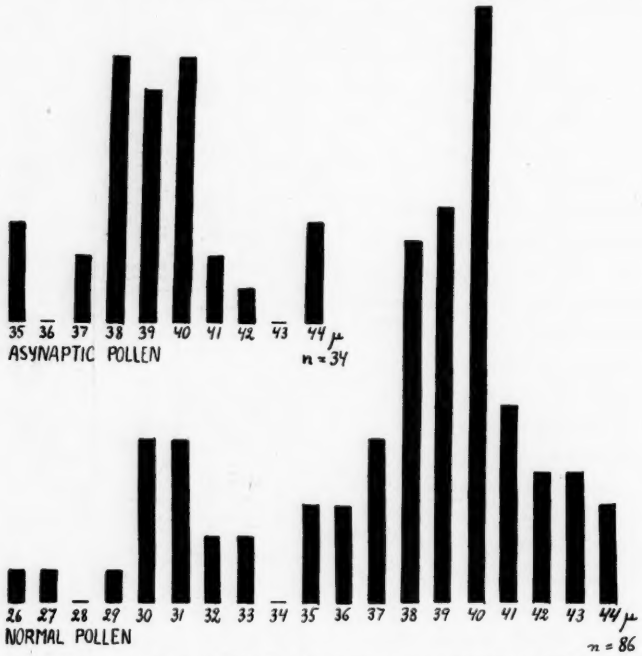


Fig. 14. Pollen length in the normal and asynaptic type.

pollen (Table 1). The map (Fig. 13) gives a summary of these conditions.

It will also be seen from the map that the asynaptic genotype is scattered over the total area of the species. Since necessarily very few specimens from each county could be examined, it is impossible to draw any far-fetching conclusions from the map. A few facts may still be pointed out although with some reservation. All specimens from San Diego are asynaptic, which makes it plausible that this locality, isolated from the rest of the distribution of the species, may contain exclusively

asynaptic types. And in the north-eastern corner of the distribution evidently asynapsis predominates (14 asynaptic specimens as against 4 normal). Normal meiosis has a cumulative district in the inner parts of California (Placer, Nevada, Yuba, Butte, Tehama and Shasta Counties) with 25 examined specimens, among which not a single asynaptic type was found. In order to get a deeper knowledge of the situation it will be necessary, however, to carry out detailed field studies.

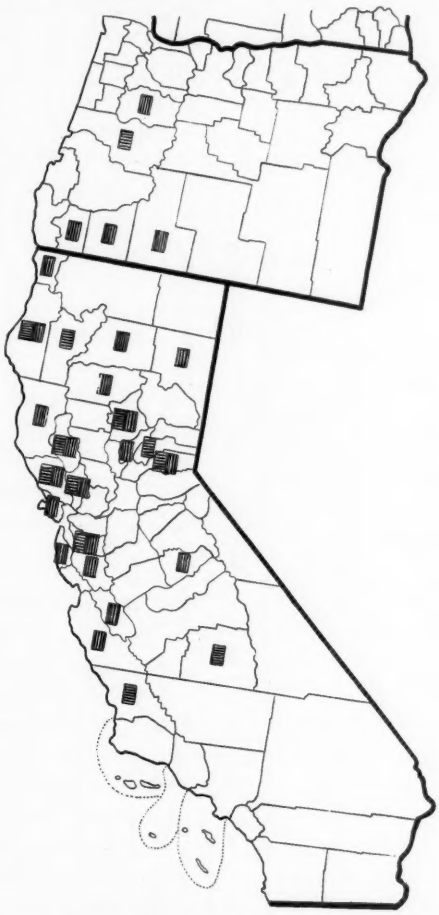


Fig. 15. The distribution of diploid and tetraploid types of *Allium amplectens*, transverse lines = diploids, longitudinal lines = tetraploids.

Pollen grains were measured from each pollen sample. It is important that identical stages of development are present in the measured samples. This condition was easily fulfilled, however, since even in dried material the nuclei of the pollen are clearly visible in acetocarmine slides. I selected for measurement the stage a few days after the first pollen mitosis, when the strongest growth of the pollen grains is past. Then both the pollen nuclei could be readily seen in most slides. In some cases it was possible even to see that the specimen had been dried during the pollen anaphase, as clearly necrotic restitution nuclei had been formed in the pollen grains.

The averages of the pollen lengths are collected in the graph

TABLE 2. *The occurrence of diploid and tetraploid forms in the different counties.*

Diploid forms			Tetraploid forms						Both diploids and tetraploids			
County No.	County	Number of specimens	County No.	County	Number of specimens	County No.	County	Number of specimens	County No.	County	Number of specimens	
											2x	4x
2	San Luis Obispo	1	3	Monterey	1	22	Yuba	1	9	Alameda	1	1
4	Tulare	2	5	San Benito	1	24	Tehama	3	12	Sonoma	2	2
10	Contra Costa	1	6	Mariposa	1	25	Shasta	5	13	Napa	1	3
21	Nevada	3	7	Santa Clara	7	28	Lassen	1	15	Lake Calif.	1	11
26	Trinity	1	8	San Mateo	2	30	Josephine	1	18	Humboldt	1	4
35	Lane	1	11	Marin	3	31	Jackson	2	20	Placer	1	1
			17	Mendocino	6	32	Klamath	1	23	Butte	1	8
			19	Del Norte	1	36	Linn	1				
	Total	9					Total	37		Total	8	30

(Fig. 14). It will at once be seen that the normal pollen grains form a bimodal curve. This indicates that the material contains not only the tetraploid forms, which in actually cytologically examined material were shown to have a pollen length corresponding to the right mode of the graph, but also diploid forms corresponding to the left mode. The curve of the asynaptic pollen, too, shows a tendency to more than one mode. In this case, however, there is too little material to allow any conclusions being drawn.

If a pollen length of 34 μ is selected as the limit between diploids and tetraploids with normal pollen, the result in the distribution of the different forms is the one represented in the map (Fig. 15) and in Table 2. It will be seen from these data that the tetraploids are very much commoner in nature than the diploids: among 84 specimens examined, 67 were tetraploid and 17 diploid. 6 counties had only diploid forms, 16 had tetraploid forms and 7 had both types.

4. THE CORRELATION BETWEEN POLLEN TYPE AND MORPHOLOGY.

Two morphological properties, plant height and pollen fertility, were plotted against pollen type. Table 3 gives the results concerning plant height. It will be seen from the table that the plant height is decidedly lower in the diploids than in the tetraploids and the asynaptic forms. On the other hand, there is no significant difference between the asynaptic plants and the normal tetraploids. In various places in the list of the herbarium specimens I pointed out that a difference in height and in general viability running in the wrong direction could be found if certain individual diploid and tetraploid specimens were compared. It is clear that the genic variation in these cases goes in the opposite

TABLE 3. *Pollen type and plant height.*

Pollen type	Plant height in cm.																		Total	Medium
	19	21	23	25	27	29	31	33	35	37	39	41	43	45	47	49	51	53		
Normal {2x	1	1	1	1	6	1	—	1	5	—	—	—	—	—	—	—	—	—	16	$27{,}8$
Normal {4x	3	2	2	7	7	7	9	5	7	7	2	2	2	2	—	—	—	3	63	$33{,}2$
Asynaptic	1	4	4	3	2	3	2	4	3	1	3	1	—	1	—	—	—	2	34	$31{,}5$

direction to the genomatic variation. This source of error is diminished if a sufficient number of individuals are collected within each group. The genic variation within the groups cannot under such circumstances conceal the difference between the groups.

In Table 4 the pollen fertility of the different pollen types is examined. In spite of the fact that some diploids had very bad pollen (in reality triploids?), the diploids turned out to have, on the average,

TABLE 4. *Pollen type and pollen fertility.*

Pollen type	Percentage good pollen											Total	Medium
	0	10	20	30	40	50	60	70	80	90	100		
Normal 2x	—	—	1	1	1	—	1	2	1	9		16	79,4
Normal 4x	1	1	2	4	9	12	7	17	7	4		64	61,7
Asynaptic	1	3	2	2	—	3	3	8	8	2		32	62,7

better pollen than the other groups. More than half of the diploid plants had more than 90 % good pollen, a fertility rarely occurring among the other types.

It must be considered of interest that a difference between the normal diploids and tetraploids could be demonstrated even by these rather crude methods. It affords a certain support to the method of classification of the pollen; it shows, in my opinion, that the division into diploids and tetraploids, made according to the pollen size, really represents valid conditions.

III. DISCUSSION.

The term *asynapsis* usually denotes a whole group of phenomena, characterized by a decreased chromosome pairing during the first meiotic division. Asynapsis can be modificative or genotypic. Temperature, age, chemical action may cause asynapsis. Evidently failure of chiasma formation is the typical response, if a certain stage of meiosis is subjected to external irritations. The genotypic asynapsis, on the other hand, may be due to lacking chromosome homology, that is the case in numerical and structural hybrids and haploids, and it may be due to a true gene action.

In all probability the case of *Allium amplectens* belongs to this last category. By studying the earlier, best known cases of such asynapsis, viz. *Zea* (BEADLE, 1930, 1933), *Datura* (BERGNER, CARTLEDGE and BLAKESLEE, 1934), *Crepis* (RICHARDSON, 1935), *Pisum* (KOLLER, 1938),

and *Nicotiana* (GOODSPEED and AVERY, 1939), it should be possible to obtain a picture of the normal course, along which the genically caused asynapsis takes place. I shall now examine to what extent the *Allium* case agrees with this normal course and to what extent deviations occur.

The chromosome pairing at zygotene is normal in the genically caused asynapsis cases. This has been actually observed in *Zea*, *Crepis* and *Nicotiana*. And this condition is even more evident in *Allium*, where the prophases are especially clear. It has been demonstrated beyond doubt that the chromosome pairing at pachytene is morphologically as intimate and complete in the asynaptic as in the synaptic types. It should be mentioned, however, that in an asynaptic *Rumex acetosa*, studied by YAMAMOTO (1934), a clear difference in zygotene pairing was observed as compared with synaptic types.

It is evident that no conclusions can be drawn concerning the formation of chiasmata from the appearance of the pachytene, if the pairing is good. This is seen in those *Allium* forms which have their chiasmata localized to the centromeric region. They have apparently quite regular pachytene pairing along the whole length of the chromosomes. And in a tetraploid species with such chiasma localisation the frequency of quadrivalents is great at pachytene and early diplotene, while it is almost nil at metaphase I. This is explained by the condition that only the pairing close to the centromeres gives rise to chiasmata (LEVAN, in the press).

According to DARLINGTON (1937 and elsewhere), the pairing which predisposes to asynapsis, involves a change in the precocity conditions of the meiotic prophase, i. e. a premature division of the chromosomes, which impedes true pairing and leads to the formation of chiasmata only in exceptional cases. The pairing that remains in such chromosome regions is therefore not due to chiasmata but to the torsion of the chromosomes.

In most earlier cases of asynapsis the suppression of chiasmata is never total, but a certain, by no means small percentage of chiasmata originates. This percentage is liable to great variation in the same individual, all values from 0 to 100 % may occur. In *Zea* an average of 3—82 % bivalents is formed in 8 plants examined, in *Crepis* about 50—70 % of the potential bivalents were realized, and in *Pisum* the corresponding values were of a similar magnitude (at diakinesis 71 %, at metaphase 54—64 %). In this respect *Allium* is very strikingly different from earlier cases, the asynapsis of *Allium* being very much more complete. One chiasma in some 500 cells is typical and the

highest frequency of chiasmata observed is 4 chiasmata in 100 cells. This corresponds to a percentage of realized chiasmata counted on the potential chiasmata of only about some 100th per cent.

The only previously described asynapsis case which in this respect is comparable to *Allium* is *Datura*, where often 24 univalents were found at metaphase I. Their behaviour was quite irregular, however, and they were not collected in any equatorial plate.

The lack of chiasmata in *Allium* at metaphase I must be considered rather remarkable, if viewed in connexion with the complete pachytene pairing. There has often been a tendency in the literature to interpret the few chiasmata which are formed in the asynaptic species as corresponding to the amount of pairing seen at pachytene. Thus DARLINGTON (1937, p. 405) writes: »Where failure of chiasmata is absolute, pachytene is also very defective. Where it appears complete, chiasmata are always formed in a proportion of nuclei, a proportion subject to great local variation». The conditions in *Allium* show, however, that it is not necessary for an apparently normal pachytene pairing to lead to the formation of any chiasmata.

A common feature in all earlier cases of asynapsis is that the spindle never acquires any command over the univalents. While the few bivalents may often be arranged into an equatorial plate, the univalents remain irregularly scattered out over the whole length of the spindle. The spindle itself behaves characteristically, it grows in length and is often bent round in the cell so that its poles approach each other (cf. the behaviour of the spindle in asynaptic *Drosophila pseudoobscura*). In *Allium* there is no trace of all this. The first division is above all characterized by the complete control of the spindle over the situation. The spindle is regular, bipolar. The univalents and the solitary bivalents are moved towards the equator and arranged into one plate or, in addition, into one or two accessory plates. The bivalents have the same type of orientation as the univalents.

The cause of this striking difference from other cases of asynapsis must be found in the behaviour of the centromeres. They must in *Allium* have the same structure both in univalents and in bivalents. They must all be polarized and capable of auto-orientation. This is no doubt the cause of the great regularity with which the first division takes place, and this also causes the difference from earlier cases of asynapsis and also the behaviour of univalents in general, which owing to lack of partners and lack of centromeric polarisation are devoid of the possibility of auto- as well as co-orientation. Ordinary univalents,

it is true, very often later on, after the separation of the bivalents, proceed towards the equator and orientate themselves into a secondary equatorial plate. This may be expressed in this way: their centromeres were unpolarized from the beginning, but later on became polarized. In *Allium*, on the other hand, the polarisation must occur very early, at the latest immediately after the disappearance of the nuclear membrane.

The cause why the centromeres in *Allium*, in spite of their polarisation, remain undivided through the first division is unexplained. It must be seen in connexion with the rhythm of meiosis, where the centromeres normally have to play a part in two nuclear divisions without being divided more than once. In the c-meiosis (LEVAN, 1939) the contrary behaviour is present, the chromosomes separate in the first division and soon after this the centromeres divide without any interkinesis despiralisation. I attributed this behaviour to the lack of pressure on the centromeres of the interior spindle, which should involve a preparation for a rapid division of the centromeres. The conditions of *Allium amplectens* argue against this assumption. No pressure of any interior spindle is present and the centromeres are still undivided.

Abruptly, while the chromosomes are being prepared for the anaphase I, the telophase starts, and the chromosomes still on the equatorial plate are included into a disciform interkinesis nucleus. No directly comparable case to this behaviour seems to be known. ROSENBERG's (1927) semi-heterotypic division has the same end-result but its course is very different.

Immediately after the interkinesis follows the second division, during which the chromosomes are less contracted than during the first division. Worth mentioning is the observation that no accessory plates, which are almost regularly present at the first division, were seen during the second division, all the centromeres being very regularly arranged in the equatorial plane. Since the chromosomes are really much larger during the second division than during the first it seems improbable that mere crowding of the plate should be the cause of the failing congression of the chromosomes in the first division. Besides that, non-congression has hardly been observed earlier in chromosomes with polarized centromeres. Instead it is found among bivalents, which, due to crowding and body-repulsion, are prevented from arranging themselves in the first metaphase plate. The situation in *Allium amplectens* goes to show that it cannot be just the conditions of the centro-

meres, but rather the special conditions of the first metaphase, which cause the formation of accessory metaphase plates.

The result of meiosis is dyad pollen with somatic chromosome sets. Apart from the first division, which did not lead to any chromosome separation, a certain agreement is noted with the deviation of meiosis, described by GUSTAFSSON (1935) in apomictic *Taraxacum* and called pseudo-homeotypic division. Its end-result is also unreduced dyads. The behaviour of the univalents, which are gathered in an equatorial plate, is ascribed to their bipolarity: »Owing to the failure of the spindle to stretch and the failure of the nuclear membrane to form, the spindle attachments of the univalents are able to divide and the chromosomes, as a result of their bipolarity, actively move towards the equatorial plane, where the halves separate» (l. c. p. 81).

From the above discussion it will be seen that the asynapsis of *Allium amplectens* is of a greatly different course from the cases of asynapsis described earlier, although it clearly belongs to the same type as *Zea*, *Datura*, *Crepis*, *Pisum* and *Nicotiana*. The regularity of the whole process makes the *Allium* asynapsis comparable to other gene-controlled deviations of meiosis. The closest resemblance exists, as mentioned above, to the monokinetic meiosis (LEVAN, 1935), where the first division occurs normally but the second is omitted. In this abnormality the mechanism is quite different, however, chiasmata being formed in a normal manner and the pre-reduced parts of the bivalents separating at the first division. The second division is lacking, no chromosome contraction and no spindle formation are seen. It is substituted by a long resting stage. The result is, generally speaking, the same as after asynapsis: pollen dyads with doubled chromosome number. Since the first division takes place, meiotic abnormalities due to autopolyploidy occur (and most monokinetic types are autopolyploid). The result of this is that the chromosome complement of the pollen varies, and in no way is so well-balanced as the asynaptic pollen. The monokinetic pollen is also distinguished from the asynaptic pollen by purely morphological characters, its chromosomes being arranged in pairs. The frequency of monokinetic pollen is very seldom exactly 100 %, often a few normal pollen grains occurred even in the most completely monokinetic types. Asynapsis, on the other hand, is almost always absolute. Only in a couple of herbarium specimens was the simultaneous occurrence of normal and asynaptic pollen observed in the same plant. And in these cases it is quite possible that the dyads were essentially of monokinetic origin.

Unfortunately I have not yet been able to study these gametic anomalies on the female side. And in *Allium amplectens* I have, owing to difficulties of cultivation, not been able to grow any progenies. The vegetative propagation is, however, so lively in *Allium* that even types with unbalanced meiosis and subsequent bad seed fertility may acquire a wide distribution in nature. The pollen size of *Allium amplectens* shows that the tetraploid is probably the maximal chromosome number occurring in nature. This indicates that if the asynaptic type is seed fertile, some regulative mechanism must be present which keeps the chromosome number down, for instance, apogamous development of the embryos.

At any rate it was demonstrated that asynaptic meiosis is widely distributed within the area of the species. The species is very multiform and evidently consists of a multitude of ecotypes more or less specialized for certain exterior conditions (for instance, the San Diego form, the Sonoma form, the Nevada form). It would be interesting to find out if the asynapsis gene in its infiltration of the species has any preference for certain morphological types. As far as can be known at present, this does not seem to be the case. Plant height, for instance, a property which is fairly characteristic of different ecotypes, exhibited a similar variation in the asynaptic types to that in the normal. Nor does the asynapsis gene seem to have any preference for certain ecological or geographical habitats. A closer study of these problems would probably give interesting results.

The fact that the asynapsis gene does not seem to have any selective value, either positive or negative, points to a vegetative or apomictic type of propagation, in which case a meiotic quality is of no importance (cf., for instance, the great distribution in nature of seed sterile triploid *Allium carinatum*).

Concerning the question of polyploidy in *Allium amplectens* the conditions are somewhat different. There is evidently present a clear difference in plant height and probably also in general viability between diploid types and tetraploids. The tetraploids are superior in the selection, they are of a commoner occurrence in nature. Conditions are often similar in other *Allium* species. The tetraploid *Allium oleraceum* is more viable than the related diploid *Allium paniculatum*. In *Allium Schoenoprasum* the diploids predominate in nature, but the only known autotetraploid is a giant form. In *Allium nutans* the tetraploids are, on an average, more robust and viable than the diploids.

It is possible that the tetraploids of *Allium amplectens* represent

the optimal stage of genomic viability of the species. It is also possible, however, that the triploid is an equally vital type to the tetraploid or even somewhat superior. Among the asynaptic types the triploids seem to be most common. All the asynaptic forms, in which the chromosome number was directly determined, were triploids, and in the graph of the pollen size (Fig. 14) the middle mode is the largest one. This might be due to an apogamous seed formation, which in the asynaptic types preserves the triploids, while among types with normal meiosis the triploids, owing to sterility, would be doomed to failure.

SUMMARY.

Allium amplectens TORR. is an endemic of the North-American Pacific Coast region. Its cytological conditions are of specially great interest since a genically controlled asynapsis is of common occurrence among its natural populations.

The first part of this paper deals with the cytology of the normal and asynaptic forms of the species. Its basic chromosome number is 7 and the chromosome morphology agrees with previously studied *Allium* species of the 7-series, with the important exception that no satellited chromosome is present. This is connected with the peculiar conditions of the nucleoli, which are formed at several chromosomes of each genome. Meiosis of normal and asynaptic forms is described. The asynaptic forms show the following interesting features: The zygotene pairing is normal, the failure of chiasma formation is nevertheless almost complete, 1 chiasma in 500 cells being formed. At the first metaphase the spindle functions normally and all the univalents are arranged at the equator. The centromeres are not divided and the metaphase goes directly over into a uninuclear interkinesis. At the second division the centromeres divide, which is followed by a normal anaphase, giving rise to pollen dyads with somatic chromosome complements. Besides the dyads there are also found single monad pollen grains, where both meiotic divisions have been omitted, due to the added action of asynapsis and monokinetic meiosis.

The second part is based on studies of herbarium material. The distribution in nature of the species is determined. Further, pollen samples from the herbarium specimens are examined and the occurrence of asynapsis as well as of diploid and tetraploid forms is recorded on the map. Finally, the asynapsis of *Allium amplectens* is discussed in relation to earlier cases of asynapsis.

LITERATURE CITED.

1. ABRAMS, L. 1923. An illustrated flora of the Pacific states Washington, Oregon and California. — Stanford University.
2. BEADLE, G. W. 1930. Genetical and cytological studies of Mendelian asynapsis in *Zea Mays*. — Cornell Univ. Agr. Exp. Stat. Mem. 129.
3. — 1933. Further studies of asynaptic maize. — Cytologia 4.
4. BELLING, J. 1928. The ultimate chromomeres of *Lilium* and *Aloë* with regard to the number of genes. — Univ. Cal. Publ. Bot. 14.
5. BERGNER, A. D., CARTLEDGE, J. L. and BLAKESLEE, A. F. 1934. Chromosome behaviour due to a gene which prevents metaphase pairing in *Datura*. — Cytologia 6.
6. DARLINGTON, C. D. 1937. Recent advances in cytology, 2nd ed. — London.
7. GOODSPEED, T. H. and AVERY, P. 1939. Trisomic and other types in *Nicotiana sylvestris*. — Journ. of Gen. 38.
8. GREENE, E. L. 1894. Manual of the botany of San Francisco Bay, being a systematic arrangement of the higher plants growing spontaneously in the counties of Marin, Sonoma, Napa, Solano, Contra Costa, Alameda, Santa Clara, San Mateo and San Francisco in the state of California. — San Francisco.
9. GREY, A. 1867. Characters of new plants of California and elswhere, principally of those collected by H. N. BOLANDER in the state geological survey. — Proc. Amer. Acad. of Arts and Sci. 7.
10. GUSTAFSSON, Å. 1935. Studies on the mechanism of parthenogenesis. — Hereditas XXI.
11. JEPSON, W. L. 1922. A flora of California, 4. — Berkeley.
12. — 1925. A manual of the flowering plants of California. — Berkeley.
13. KELLOGG, A. 1861. *Allium attenuifolium* (KELLOGG). — Proc. Calif. Acad. nat. Sci. 2.
14. KOLLER, P. C. 1938. Asynapsis in *Pisum sativum*. — Journ. of Gen. 36.
15. LEVAN, A. 1931. Cytological studies in *Allium*. A preliminary note. — Hereditas XV.
16. — 1932. Cytological studies in *Allium*, II. Chromosome morphological contributions. — Hereditas XVI.
17. — 1935 a. Cytological studies in *Allium*, VI. The chromosome morphology of some diploid species of *Allium*. — Hereditas XX.
18. — 1935 b. Zytologische Studien an *Allium Schoenoprasum*. — Hereditas XXII.
19. — 1937. Cytological studies in the *Allium paniculatum* group. — Hereditas XXIII.
20. — 1938. A triploid asynaptic *Allium amplectens* from California. — Nature 142.
21. — 1939. The effect of colchicine on meiosis in *Allium*. — Hereditas XXV.
22. MATSUURA, H. 1938. Chromosome studies in *Trillium kamtschaticum* PALL. VI. On the nucleolus-chromosome relationship. — Cytologia 6.
23. MCCLINTOCK, B. 1934. The relation of a particular chromosomal element to the development of the nucleoli in *Zea Mays*. — Z. f. Zellf. u. mikr. Anat. 21.
24. MUNZ, P. A. 1935. A manual of southern California botany. — Claremont.
25. PIPER, C. V. 1906. Flora of the state of Washington. — Contr. U. S. Nat. Herb. 11.

26. REGEL, E. 1875. Alliorum adhuc cognitorum monographia. — Petropolis.
27. RESENDE, F. 1937. Über die Ubiquität der SAT-Chromosomen bei den Blütenpflanzen. — Planta 26.
28. RICHARDSON, M. M. 1935. Meiosis in *Crepis*. II. Failure of pairing in *Crepis capillaris* (L.) WALLR. — Journ. of Gen. 31.
29. ROSENBERG, O. 1927. Die semiheterotypische Teilung und ihre Bedeutung für die Entstehung verdoppelter Chromosomenzahlen. — Hereditas VIII.
30. TORREY, J. 1856. Description of the general botanical collections of the route near the 35th parallel explored by Lieut. A. W. WHIPPLE 1853—1854. — U. S. War Dept. Rep. on explorations and surveys for a railroad from the Mississippi River to the Pacific Ocean, 4.
31. UPCOTT, M. 1939. The external mechanics of the chromosomes. VII. Abnormal mitosis in the pollen-grain. — Chromosoma 1.
32. WOOD, A. 1868. A sketch of the natural order Liliaceae as represented in the flora of the states of Oregon and California with special reference to the plants collected in an excursion along our Pacific Coasts A. D. 1866. — Proc. Acad. nat. Sci. Philadelphia.
33. YAMAMOTO, Y. 1934. Reifungsteilungen bei einer asynaptischen Pflanze von *Rumex acetosa* L. — Bot. Zool. 2.

CONTENTS.

Introduction 353

I. Cytological studies 355
1. Material and methods 355
2. Somatic chromosomes 356
3. Meiosis of the normal type 357
4. Meiosis of the asynaptic type 359
5. The mitosis of the pollen grain 365
a. The normal type 365
b. The asynaptic type 365
6. The nucleoli 368

II. Herbarium studies 371
1. Historical sketch 371
2. Herbarium material examined 375
California 375
Oregon 379
Washington 379
3. Plant-geographical survey 379
4. The correlation between pollen type and morphology 385

III. Discussion 386
Summary 392
Literature cited 393

A HYBRID BETWEEN TRITICUM TURGIDUM AND AGROPYRON JUNCEUM

BY *G. ÖSTERGREN*
INSTITUTE OF GENETICS, LUND, SWEDEN

BOTH Russian and Canadian workers have successfully crossed wheat with *Agropyron junceum* (TSITSIN, cited by VERUSHKINE, 1935, p. 8; JOHNSON, 1938, p. 240).

The female parent of the hybrid studied by me was *Triticum turgidum* L., »Rivet's Bearded». Pollen was obtained from a wild population of *Agropyron junceum* (L.) P. B. growing at Råå in South Sweden. 245 cross-pollinated flowers gave 38 seeds (15,5 %). The seeds were germinated in petri-dishes containing sand and water. Three plants were obtained. Some more seeds germinated, but the resulting seedlings died at an early stage.

Only one of the plants flowered in 1939 (it had been germinated in the autumn of 1938). Two plants germinated in the spring of 1939 have not flowered yet.

The morphology of *Triticum turgidum* × *Agropyron junceum* seems to be rather interesting, this hybrid being more similar to wheat than most *Triticum-Agropyron* hybrids described in literature. This is especially true of the spikes (Fig. 1). These statements are based on the general appearance of the hybrid. The inheritance of the individual characters has as yet been observed only in a few cases. The

	Configuration		Number of cells	
	Univalents	Bivalents	Fixation a	Fixation b
	14	7	1	2
	16	6	4	7
	18	5	8	11
	20	4	8	3
	22	3	2	2
	24	2	1	—
	28	0	1	—
Average a	19,12	4,44	Total 25	25
» b	17,68	5,16		
» of a and b	18,4	4,8		

beards of the wheat parent are recessive (although not completely). The pubescence of the spikelets is dominant. *Agropyron junceum* has its nodes concealed within the vaginae, and so has the hybrid. The leaves of *A. junceum* have on their underside a sub-epidermal layer of mechanical tissue. This character is recessive. It is not known as yet

Fig. 1. Spikes of the material. From left to right: *Triticum turgidum*; the F_1 hybrid; *Agropyron junceum*.

if the hybrid is perennial. Its morphology will be treated more in detail in a later paper (e. g. in conjunction with the descendants of the hybrid if such can be obtained).

The root tips and the anthers were fixed in chrome-acetic-formalin. The anthers were prefixed in 96 % alcohol. The preparations were stained with gentian violet. The three hybrid plants had $2n = 28$, like both parents. Two fixations of the plant which flowered gave the

frequencies of univalents and bivalents at first metaphase tabulated above (p. 395).

First metaphase was observed also in an E.M.C. The configuration could not be analysed, but seemed to be similar to those observed in the P.M.C:s.

Most chiasmata are terminal, but some are subterminal. Most bivalents are rod-shaped. In the cell presumably having 0_{II} the configuration is not quite clear. In a few cells some possible quadrivalents were observed, but these might perhaps be composed of two bivalents.

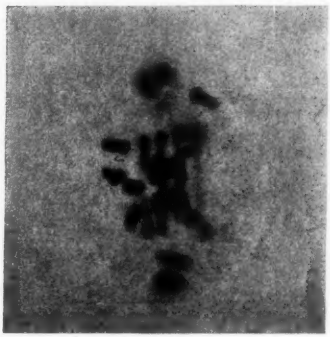


Fig. 2. Microphotograph of a cell with 7_{II}. — × 1400.

Assuming that the chromosome pairing is not reduced by other causes than an incomplete homology between the chromosomes, the conclusion can be drawn that *A. junceum* is not completely autopolyploid. This observation does not prove with certainty, however, that the quadrivalents observed by me in *A. junceum* (ÖSTERGREN, 1940) are due to segmental interchange.

Autosyndesis between the A- and B-genomes of wheat can probably account only for a minor part of the pairing found in this hybrid. The chromosome pairing in haploid *T. durum* was studied by KIHARA (1936). The chief causes of the pairing in my hybrid should then be autosyndesis between the *junceum*-chromosomes and allosyndesis. Allosyndesis or autosyndesis may prevail, or both may occur to about the same extent.

In two pollen tests, containing more than 1000 grains, not a single good grain was found.

The relatively pronounced similarity of this hybrid to wheat may be of interest from a practical point of view. Its high sterility does not make it very probable, however, that an F_2 can be obtained. Perhaps it may give rise to an amphidiploid.

LITERATURE CITED.

1. JOHNSON, L. P. V. 1938. Hybridization of *Triticum* and *Agropyron*. IV. Further crossing results and studies on the F_1-hybrids. — Canad. Journ. of Research, Sect. C, Vol. XVI, pp. 417—444.

2. KIHARA, H. 1936. Ein diplo-haploides Zwillingspaar bei *Triticum durum*. — Agric. and Hortic., Bd. 11, pp. 1425—1434.
3. VERUSHKINE, S. M. 1935. On the hybridization of *Triticum* by *Agropyrum*. — Saratov.
4. ÖSTERGREN, G. 1940. Cytology of *Agropyron junceum*, *A. repens* and their spontaneous hybrids. — Hereditas XXVI, pp. 305—316.

STUDIES ON THE SIGNIFICANCE OF POLYPLOIDY

IV. OXYCOCCUS

BY *O. HAGERUP*
BOTANICAL MUSEUM, COPENHAGEN

ANYONE who has had the opportunity of studying the growths of *Oxycoccus*, which adorn the *Sphagnum* bogs of our country, will have noted that this small shrub varies greatly; thus, for instance, its fruits are of different shape, size, and colour. Very conspicuous, also, is the variation in thickness (Figs. 2—3), shape, and size of the leaves (Figs. 7—9), and the manner in which the margin is recurved. Often the leaves are almost vertically erect, but they may also be horizontally expanded (Fig. 1). The flowers, too, differ in a number of characters, of which their number and the pubescence of the stalk, in particular, are of taxonomic value.

If, in addition, one has the opportunity of examining abundant material derived from ecologically different localities and from other countries, the various forms will be found to differ also in ecological and geographical respects.

These characteristic differences in morphological, ecological, geographical, and systematical characters are combined in an interesting way with equally characteristic cytological features, which will be described in detail below, as far as the available material permits.

My material consisted of a fairly large collection of dried plants belonging to the Botanical Museum of the University of Copenhagen and collected in different countries; but in addition I had collected an abundant living material in several bogs in the vicinity of Copenhagen and in Greenland near 66° N. lat.

It is of great value that several investigators have collected cytological material for me of foreign types, difficult to obtain. For this reason I owe special thanks to Dr. A. E. PORSILD, Canada, and to Mr. G. THORLÁKSSON, B. Sc., who fixed *O. microcarpus* in North Iceland.

The chromosomes are excellently stained by the method of FEULGEN.

The taxonomy has been well treated by SAMUELSSON, M. P. PORSILD, and A. E. PORSILD, whose views and nomenclature I adopt in the following account. Most of the floristic manuals, however, are almost useless in an attempt to ascertain the geographical conditions,

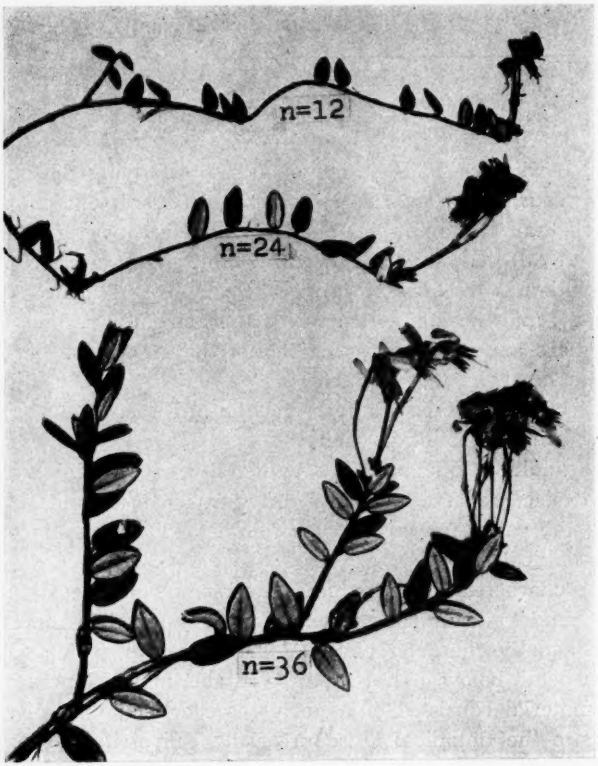


Fig. 1. Top: *O. microcarpus* from N. Iceland (66° N. lat.). Middle: *O. quadripetalus* var. *microphyllus* from West Greenland (66° N. lat.). Base: *O. gigas* from the Lyngby bog near Copenhagen. — Natural size.

partly because of the confusion of the nomenclature, and partly because the authors have not been sufficiently interested in the varieties. On that account it is possible at present to give only the main features of the distribution of the various forms; thus the geographical conditions are badly in need of a further elucidation, so that the distribution of the different forms may be mapped.

Oxycoccus microcarpus TURCZ. (n = 12). — In nearly all its parts the plant proves to be the smallest representative of the genus. That it should be considered an independent species has already been shown by SAMUELSSON, who had a large material at his disposal, both from Scandinavia and the Central European Alps, where the plant is widely distributed and is distinctly separated from the other *Oxycoccus* forms, with which it rarely forms hybrids.

Apart from its small size, *O. microcarpus* is characterized by the shape of its leaves, but especially by being the only species with a glabrous flower stalk. In addition, the plant is recognisable by means of several other characters, which have been described in detail by SAMUELSSON. Moreover, its chromosome number is n = 12 (Fig. 7), a number not found in any of our other *Oxycoccus* forms, whereas this is the chromosome number most commonly found within the *Bicornes*.

Finally, it may be mentioned that it flowers one or two weeks earlier than *O. quadripetalus*.

Of special interest, however, are MELIN's investigations of the ecological conditions of the plant. MELIN found that *O. microcarpus* is very exclusive in the choice of its habitat, which differs from those of the other forms, in that it is only found in the driest *Sphagnum* bogs characterised by the occurrence of *Sph. fuscum*, *Hypnaceae*, and other xerophilous species.

The geographical distribution also is of interest. Thus, with regard to its distribution in North America, A. E. PORSILD writes as follows (1938, p. 117): »In sub-arctic *Sphagnum*-bogs only. From Alaska to the east shore of Hudson Bay penetrating but a short distance north to the limit of trees. From the Yukon Territory south through the mountains of British Columbia and Alberta. Throughout northern Alaska and the Northwest Territories *O. microcarpus* is the only representative of the genus».

The same thing may be said of the occurrence of the plant in Europe and Asia. It is circumpolar and is the species found farthest north.

In Iceland the only *Oxycoccus* species that has been found is *O. microcarpus*, which is known from a few scattered localities, especially along the north coast of the island. It is therefore all the more curious that the species has not been recorded from the nearest part of the east coast of Greenland or from the Faroes.

The species is widely distributed in northernmost Scandinavia. It decreases rapidly southward, but is recorded with certainty from the

areas round the Baltic as far as the North German moorlands (SAMUELSSON, 1922, p. 258). It has not yet been found in Denmark, but should be more carefully searched for, since it is found in the neighbouring countries both to the north and to the south.

SAMUELSSON (pp. 257—58) has also found the plant in several places in the mountains of Central Europe; thus *O. microcarpus* has a similar geographical distribution to that of the bisexual *Empetrum hermaphroditum*.

The southern limit of the distribution of the species is almost

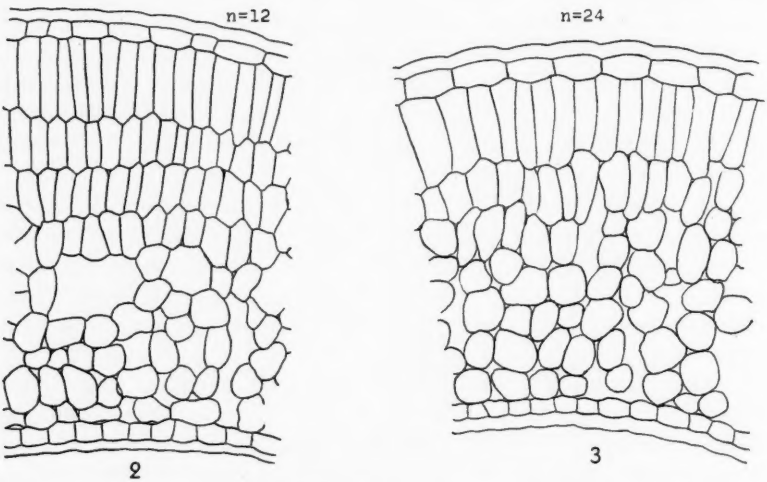


Fig. 2. Cross section of leaf of *O. microphyllus* from N. Iceland. — × 220. — Fig. 3. Cross section of leaf of *O. quadripetalus* from Denmark. — × 220.

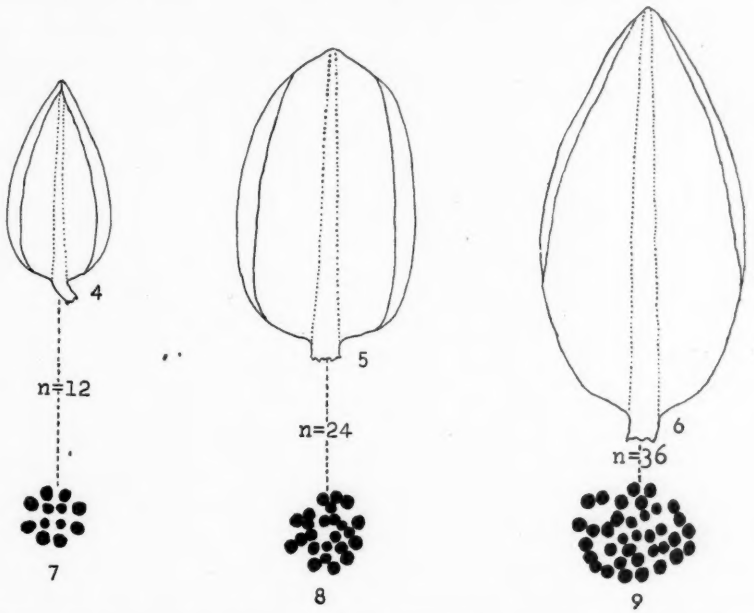
entirely unknown. However, in the Botanical Museum of this city there is a specimen of *O. microcarpus* (from »Flora Romaniae exsiccata, No. 1085 b») collected in Distr. Ciuc in Transylvania, 1050 m. above sea-level. This finding so far south elicits a desire to investigate the distribution of the species in all European countries. For this purpose the floristic manuals should start from SAMUELSSON's and PORSILD's conception of the species; thus our desire is justified both by cytological, ecological, and geographical conditions.

Oxycoccus quadripetalus GILIB. var. *microphyllus* (LANGE) M. P. PORSILD. (n = 24). — Very similar in habit to *O. microcarpus* (Fig. 1), for which reason the two forms have mostly been confused, so that it

is now impossible to unravel their distribution by means of the literature.

It was only by M. P. PORSILD's investigations (1930) that the taxonomy of this variety was elucidated so that it may now be distinguished from the other small *Oxycoccus* forms.

That each of these forms belongs to its particular species is evident,



Figs. 4—6. Leaves (seen from the under side) of *O. microcarpus* (Fig. 4) from Iceland; *O. quadripetalus* var. *microphyllus* (Fig. 5) from W. Greenland (66° N. lat.); *O. gigas* (Fig. 6) from Denmark. — × 8. — Below the leaves the corresponding chromosomes in Metaphase I. Fig. 7 from Iceland, Figs. 8—9 from Denmark. — × 2500.

among other things, from the fact that the flower stalk is densely pubescent in var. *microphyllus*, which is further characterised by a different leaf shape (Figs. 4—5) and by various other characters, recorded by PORSILD. Finally, each of the two small forms has its particular chromosome number, n = 24 in var. *microphyllus* (Fig. 8), which I found in individuals from three different localities in nothern Seeland. However, var. *microphyllus* from Greenland is not quite identical with the Danish one, and it should be ascertained whether

they have the same chromosome number. The Greenland plant has a short style and pollinates itself (M. P. PORSILD), the species thus having a tendency to develop pure lines in northern localities.

The plant is not found either in the Faroes, Iceland, or East Greenland, while it is recorded from some scattered localities along the west coast of Greenland from the southernmost point (c. 59° N. lat.) right up to a little north of the Polar circle (68° 41′ N. lat.). From this coast its range extends across to North America, of which A. E. PORSILD says as follows (1938, p. 117): »On the Labrador coast from Strait of Belle Isle north to 56° 16′ N. Also in Newfoundland and the Gulf of St. Lawrence region. South of Newfoundland and the Labrador coast gradually merging into the preceding» (= *O. quadripetalus*).

In Denmark the species has been found in many places; it is almost as common as the main species. Often the two forms grow together, and if so, they are evenly intermixed or remain fairly isolated in small growths. However, pure growths of one or the other species may also be found, for instance, in the Bøllemosen near Copenhagen, where a large isolated pure culture of var. *microphyllus* is found.

That the Danish forms differ somewhat ecologically may be seen when they grow near each other but in isolated patches, as is the case in the Lyngby bog near Copenhagen. For, in such instances, var. *microphyllus* behaves in the same exclusive manner as *O. microcarpus* and chooses the highest and driest *Sphagnum* tufts, being more xerophilous than *O. quadripetalus*. It may even rise to the level of *Calluna* and *Empetrum*, but does not tolerate too much shade from other plants. On the other hand, it does not thrive in the immediate neighbourhood of water (as the main species does).

In its natural habitats the plant is recognisable in that it covers but a small part of the substratum, which is due in the first place to the very small size of the leaves, but especially to the almost erect position of the greater number of the leaves as in true light plants. And in the flowering period the plant is especially conspicuous because the very small flowers are mostly single and of a very dark colour.

Var. *microphyllus* differs from *O. quadripetalus* in all these characters, and even more distinctive properties might be mentioned (such as the size of the fruit, coloration, etc.); on seeing the two forms together, one's first impression, therefore, is that they are two different species.

There are, however, a few weighty reasons against this very

natural view; firstly, a typical *microphyllus* shoot may sometimes be found to have developed as a lateral shoot from another shoot distinctly belonging to *O. quadripetalus*.

Moreover, it is of decisive importance that these two forms have the same chromosome number, viz. n = 24. I therefore prefer to adopt the view held by M. P. PORSILD that *microphyllus* is only a variety.

As regards the geographical distribution, very little is known in addition to what has been stated above; and unfortunately only scanty and unreliable information can be derived from the literature. Although, perhaps, we are only concerned with a variety, its ecological as well as its geographical conditions seem to be well worth a closer investigation. On the whole, however, var. *microphyllus* appears to be a more northerly type than the main species. Its southern limit its entirely unknown.

Oxycoccus quadripetalus GILIB. (n = 24). — This name denotes the »main species», which is so well known that there is no need of describing it in detail here. Compared with the two preceding species it is typically much larger — even though transitional forms to var. *microphyllus* are of common occurrence. The leaves are, as a rule, large; many of them are horizontally expanded, and accordingly it tolerates a higher degree of shade than the small-leafed arctic forms, which require much light.

Its need of a certain degree of moisture is not so marked either as, for instance, that of *O. microcarpus*; it can therefore thrive right out to the open water, and does not perish until it is submerged. It extends towards dry land until it is shaded by, for instance, *Calluna* and *Empetrum*; it will not thrive, or will soon die, in the shadow of trees (e. g. *Betula*).

It would be of interest to establish its northern limit and to compare it with corresponding conditions of the small-leafed forms. It is not found either on the Faroes, in Iceland, or in East and West Greenland, but it is of common occurrence in North America, Europe, and Asia, where it decreases considerably in frequency towards the south. Its distribution in Denmark has been mapped by BÖCHER.

O. quadripetalus is tetraploid (n = 24), and in this connection it should be mentioned that both geographically and ecologically the plant has thus a higher amplitude than *O. microcarpus* (n = 12).

Finally, it should be mentioned that between the three *Oxycoccus* forms referred to above, there is no difference in the size of stomata or pollen grains. Nor did I observe any differences in the anatomy

of the wood, whereas the leaves are often relatively thicker in *O. microcarpus* (see Figs. 2—3).

Oxycoccus gigas (=*O. microcarpus*×*O. quadripetalus*?). (n=36). — At first sight this form bears so great a resemblance to the ordinary *O. quadripetalus* that it may be difficult to distinguish them from each other without the use of a microscope. But if one has a sufficiently large material at one's disposal, *O. gigas* as a rule proves to be larger than the three *Oxycoccus* forms mentioned above; and many specimens grow to a size that *O. quadripetalus* will never attain; an individual from Finland even bore leaves measuring 0,7 × 1,5 cm.

Fig. 1 shows a specimen of the average size, and Fig. 6 a leaf; the shape of the leaf often differs somewhat from that of the other species.

The flowers are large, several together, and have long, hairy stalks. Fig. 1 further shows a characteristic feature, viz. that *O. gigas* develops vertical shoots whose leaves are more or less distinctly horizontally expanded.

My living material was collected in the Lyngby bog near Copenhagen, where the plant grows in abundance and shows a remarkable power of forming continuous carpets, which on account of the erect shoots and its entire mode of growth reminds one to a certain extent of *Vaccinium vitis idaea*. Often the carpets are so dense that only little or no space is left for other phanerogams, and even the mosses may be ousted by its shadow. The other *Oxycoccus* species, however, do not as a rule entirely cover the moss tufts, across which they spread passively, and only a minority of the shoots grow upwards. The stems are comparatively short, and the whole plant so robust that it acquires a different appearance.

Ecologically, too, *O. gigas* is quite unique, of all the forms it is the one that can endure the highest degree of drought; and it is often met with in the shape of dense, erect shrubs on the top of the moss tufts of the bog. It is also fairly well equipped against shade and can thrive below solitary trees (e. g. *Betula*); in such cases its leaves are horizontally expanded and grow big and broad. All the other forms tolerate only very little shade.

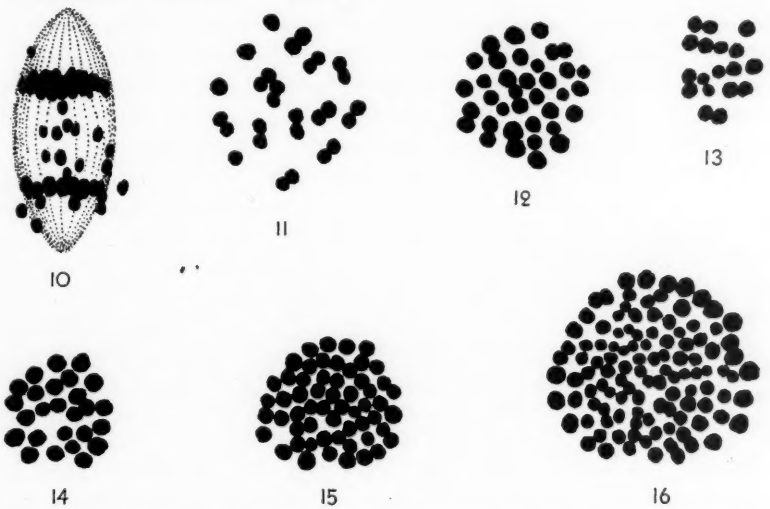
In Denmark the plant has been found as a rarity both in Jutland and on Seeland. Further, specimens from Finland and East Prussia are found in the Botanical Museum of Copenhagen. Otherwise the geographical distribution of the plant is unknown, but it would be of interest to have the matter cleared up.

Judging from the morphological and ecological conditions, it

would be natural to assume that *O. gigas* is a polyploid. And that this is actually the case will be shown more in detail below.

In the Lyngby bog near Copenhagen meiosis takes place at the end of May and the beginning of June, when the winter bud begins to grow and open, but before the flower bud has left it.

During meiosis only the first stages are fairly regular; in metaphase I (Fig. 9), however, the chromosomes are situated in such a way that it is possible to count them and to ascertain that the number is 36. Accordingly, *O. gigas* is hexaploid.



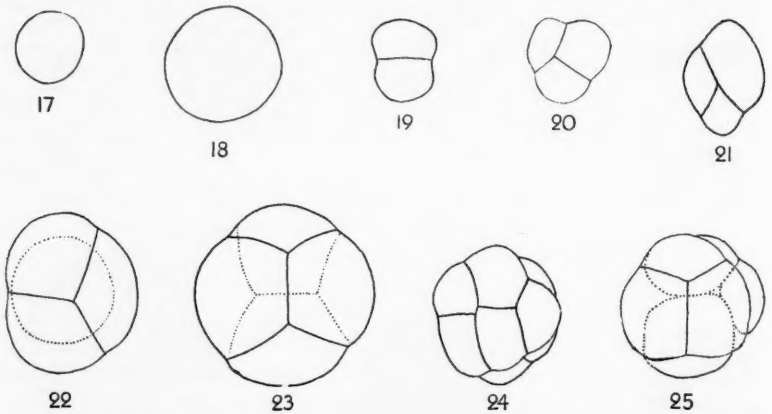
Figs. 10—16. *O. gigas*. Fig. 10, Anaphase I. Figs. 11—16, Metaphases from the first division in different microspores. In Fig. 11, n = 28; Fig. 12, n = 36; Fig. 13, n = 18; Fig. 14, n = 26; Fig. 15, n = 56; Fig. 16, n = 112. — × 2880.

After metaphase I the division stages become more and more irregular, as if the plant were a hybrid. Thus Fig. 10 shows an anaphase, in which it will be seen that the chromosomes do not keep company; and a greater number of them do not enter the two daughter nuclei, but place themselves entirely outside; in the later stages the chromosomes gather in larger or smaller groups and give rise to the formation of dwarf pollen, the size of which will depend on the number of chromosomes contained in its nucleus.

In still later stages, meiosis is so irregular that the number of the chromosomes cannot be ascertained, and only by means of FEULGEN's

reaction is it possible to follow the further development of the process. In consequence of these irregularities, it frequently happens that each PMC does not form four pollen grains, as is the case in all the other species. And if we examine the contents of a young anther, we shall find, in addition to some apparently normal tetrads, many other »tetrads» which may contain 1—10 pollen grains (Figs. 17—25).

The chromosome sets of such irregular pollen grains (Figs. 17—25) may be examined by means of a careful staining with hæmatoxylin. The metaphase must then be looked for during the first division in the microspore. This division takes place when the flower bud is



Figs. 17—25. *O. gigas.* Abnormal »tetrads» with different numbers of pollen grains. — × 850.

half-developed; it is then 2—3 mm. long, which stage may be found at the beginning of June.

As might be expected, the pollen grains (Figs. 11—16) prove to contain a greatly differing number of chromosomes. In some of the figures it is distinctly seen that, as is often the case in polyploid cells, the chromosomes have a tendency to gather in groups of two, three, or more.

Fig. 13 shows that a small pollen grain may contain 18 chromosomes, and Fig. 16 shows 112 chromosomes from a large pollen grain; it may also be possible to find all the figures from 1 to 4 × 36 in the metaphases of the pollen grains, which are mostly regular and fairly easy to count.

All these strange pollen grains do not come into function. Even

before the flower opens, they die, and the anthers then contain merely pulpy remains and resorbed pollen grains. When the flower opens, the anthers are empty, and no pollen will adhere to the numerous bees which all day suck honey from the multitude of flowers that adorn the Lyngby bog in the month of June.

It is true that, after the flowering, fruits develop in the other *Oxycoccus* species with normal pollen in the Lyngby bog. However, if we examine the large, luxuriant tufts of *O. gigas*, only few fruits will prove to continue their growth, the majority of them will soon stop growing and wither. But there always remain some which attain a considerable size; though they contain no embryo. I found embryo-sacs in the flowers examined; but the nucellus was almost entirely reduced. Germinated pollen (from the other species) was not observed by me either.

On the other hand, (Mycorrhiza?) fungi often penetrated down through the style, soon surrounding the barren seeds with a thick layer of hyphae.

Thus, as far as I have been able to observe, seed capable of germinating does not seem to develop in *O. gigas*. That it may nevertheless take place in exceptional cases is a hypothesis that cannot be entirely rejected.

Accordingly, the plant does not seem to have sexual reproduction; and how it is dispersed from place to place, is a mystery. However, the cytological conditions would seem to indicate that *O. gigas* is possibly a hybrid between a species with $n = 12$ and another with $n = 24$. If this is correct, it may have arisen several times and in different places, where it has then held its own and spread vegetatively. An argument against this supposition, however, is that at present no *Oxycoccus* with $n = 12$ is found, for instance, in the Lyngby bog nor in Denmark at all. But such a species (*O. microcarpus*) may very well have grown there in subglacial times, and its hybrid with the subsequently immigrated *O. quadripetalus* ($n = 24$) may have held its own up to the present day — thanks to the good ecological equipment which it possesses as a polyploid — in connection with its great power of vegetative propagation.

SUMMARY.

1) The genus *Oxycoccus* includes several closely related forms, whose chromosome numbers constitute a polyploid series, viz. $n = 12$, $n = 24$, and $n = 36$.

2) n = 12 in *O. microcarpus*, the species found farthest north, and which also differs ecologically from *O. quadripetalus*. Cf. further SAMUELSSON (1922).

3) n = 2 × 12. *O. quadripetalus* is the most commonly occurring species and is present in the greatest numbers. Var. *microphyllus* is its outpost to the north (Greenland).

4) n = 3 × 12. The newly discovered *O. gigas* (= *O. quadripetalus* × *O. microcarpus*?) is hexaploid. It does not produce germinable pollen, and is sexually sterile. For further details, see pp. 406—409.

5) Accordingly, the polyploid series dealt with here attains its optimum at tetraploidy. With higher chromosome numbers sexual reproduction ceases. The same thing applies to the genus *Deschampsia* (HAGERUP, 1939).

LITERATURE CITED.

1. BÖCHER, T. W. 1937. Om Udbredelsen af *Ericaceae, Vacciniaceae* og *Empetraceae* i Danmark. — Bot. Tidsskr. 44, pp. 18—19.
2. HAGERUP, O. 1939. Studies on the Significance of Polyploidy. III. *Deschampsia* and *Aira*. — Hereditas XXV, pp. 185—192.
3. MELIN, E. 1917. Studier över de norrländska myrmarkernas vegetation. — Uppsala.
4. PORSILD, A. E. 1938. The Cranberry in Canada. — The Canadian Field-Naturalist. Vol. LII, p. 116.
5. PORSILD, M. P. 1930. Stray Contributions to the Flora of Greenland. I—V. — Medd. om Grönland. 77, 1, pp. 38—42.
6. ROZANOVA, M. A. 1934. A Survey of the Literature on the Genera *Vaccinium* and *Oxycoccus*. — Bull. Applied Bot., Genet. and Plant Breeding. Serie 8, No. 2.
7. SAMUELSSON, G. 1922. Zur Kenntnis der Schweizer Flora. 14. — Vierteljahrsschr. Naturf. Ges. Zürich. Bd. 67, p. 256.

DIE MEIOSIS BEI HAPLOIDEN PFLANZEN VON GODETIA WHITNEYI

VON *ARTUR HÅKANSSON*
LUND

(With a Summary in English)

DAS Material zu vorliegender Untersuchung stammt von Dozent GUNNAR HIORTH, Landwirtschaftliche Hochschule in Ås, Norwegen, der es mir in freundlicher Weise zwecks zytologischer Untersuchung zur Verfügung gestellt hat. Es wurden ziemlich viele Mutationen untersucht, die aus Inzuchtlinien von *Godetia Whitneyi* wie auch aus einigen von *G. amoena* erhalten worden waren. Hier sollen die Ergebnisse meiner Studien über Haploide mitgeteilt werden, von denen in den Kulturen mehrere Pflanzen angetroffen worden sind.

Die untersuchten Blütenknospen sind in NAWASCHINs Flüssigkeit fixiert worden. Sie wurden eingebettet und am Mikrotom geschnitten. Die Färbung erfolgte mit HEIDENHEINs Eisenhämatoxylin. Die gemachten genetischen Angaben wurden mir von Dozent HIORTH freundlichst übermittelt. Meine *Godetia*-Untersuchungen sind mit Unterstützung des BOKELUND-Fonds der Universität Lund ausgeführt.

Zwei haploide Pflanzen wurden untersucht, G. 4359/220 (Fixierungsnr. 211) und G. 2930/132 (Fixierungsnr. 48). Erstere ist nach Bestäubung mit röntgenbehandeltem Pollen, letztere spontan entstanden. Ausserdem erwies sich eine *G. amoena*-Pflanze als haploid. Es war dies G. 8474/581 (Nr. 214), aber Meiosisstadien konnten hier nicht untersucht werden.

Die Meiosis ist an zahlreichen Haploiden studiert worden (siehe IVANOV, 1938). Ihr Verlauf wird von verschiedenen Forschern verschieden geschildert, was zum Teil auf das Material zurückzuführen ist, aber teilweise auch auf die Interpretation desselben, wie aus den Arbeiten über die Meiosis haploider *Oenothera* hervorgeht. Besonders schwierig ist es die Bewegungen der univalenten Chromosomen nach der Diakinese klarzulegen. Bei *Godetia* wird die Serienkombination der beobachteten Stadien in der Entwicklung dadurch erleichtert, dass die Pollenfächer in durch steriles Gewebe getrennte, kleinere Abteilungen aufgeteilt sind. Die PMZ in zwei angrenzenden Abteilungen

zeigen grössere Unterschiede in der Entwicklung als die PMZ in ein und derselben Abteilung.

Diakinese. — Die Diakinese zeigt 7 Univalente, die deutlich längsgespalten sind (Fig. 1 *a*). Auch im zunächst vorhergehenden Stadium, wo die Chromosomen nicht so stark kontrahiert sind, erkennt man die Chromatiden sehr deutlich. Zwei Nukleolen sind bisweilen im Diakinesekern.

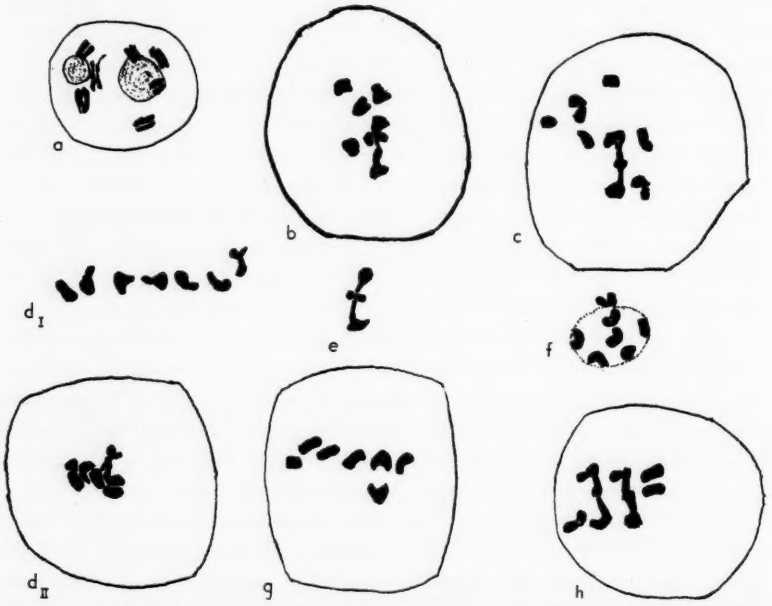


Fig. 1. — *a*: Diakinesekern. — *b*: PMZ mit Bivalent. — *c*: PMZ mit Bivalent und fragmentiertem Chromosom. — d_I und d_{II}: PMZ mit Kernplatte und die Chromosomen dieser Platte. — *e*: Bivalent mit subterminalem Chiasma. — *f*: Ein Chromosom ist ausserhalb der Spindel. — *g*: PMZ mit Ringbivalent. — *h*: PMZ mit zwei Bivalenten.

Bivalente. — Kaum 1 % der PMZ enthalten ein Bivalent. Dieses war stets in der Längsrichtung der Kernspindel normal orientiert. Es hatte ein Chiasma, das gewöhnlich terminal (Fig. 1 *b*), seltener subterminal (Fig. 1 *e*) gelegen ist. Ein einzigesmal wurden zwei Bivalente beobachtet, von denen das eine deutlich heteromorph war (Fig. 1 *h*). Im allgemeinen sind indessen die beiden gepaarten Chromosomen einander sehr ähnlich (andere Beispiele für Heteromorphie sind jedoch in den Fig. 1 *e* und 3 *a* zu sehen). Einige wenige Male wurde ein ring-

förmiges Bivalent mit zwei terminalen Chiasmata beobachtet (Fig. 1 *g*). Dass die Frequenz der Bivalentbildung von ausserhalb der Chromosomen liegenden Faktoren beeinflusst wird, darauf deutet, dass während in den meisten Kleinfächern keine PMZ ein Bivalent aufweist, in anderen zuweilen eine, aber auch zwei oder drei PMZ ein solches enthalten. Einige Forscher haben der Ansicht Ausdruck gegeben, dass die bei Haploiden beobachteten Bivalente keine echten solchen seien, sondern auf der Verschmelzung von heterochromatischen Teilen oder anderem beruhten. Aber die Orientierung der Centromeren in der Kernspindel und das Vorkommen von subterminalen Chiasmata sprechen natürlich dafür, dass es sich um eine wirkliche Chromosomenpaarung handelt, die ihre Erklärung im Vorkommen von duplizierten Stücken im haploiden Genom bekommt.

Es ist bei den meisten untersuchten Haploiden eine Bivalentbildung konstatiert worden, auch in solchen mit der »Grundzahl». Der Prozent PMZ mit einem Bivalent ist bei verschiedenen Haploiden sehr ungleich. Auch innerhalb derselben Art kann er variieren, so bei *Oenothera franciscana*, wo in ein paar Knospen fast alle PMZ ein Bivalent hatten, während in einem anderen »most of the cells failed to show» ein Bivalent (EMERSON, 1929).

Metaphase und Anaphase. — Nach dem Verschwinden der Membran des Diakinesekerns liegen bei di- und polyploiden Godetien Bivalente und Multivalente etwas zerstreut, aber sie ordnen sich schnell zu einer Metaphaseplatte. Dieses Stadium währt lange Zeit, worauf die Anaphase schnell stattfindet. Zufällig vorkommende univalente Chromosomen sind entweder in der Platte oder näher dem einen Pol beobachtet worden. Bei den haploiden Godetien verhalten sich die seltenen Bivalente wie bei den Diploiden, aber die Anwesenheit von zahlreichen Univalenten gibt ein sehr variierendes Bild dieser Stadien.

Die Univalente sind ziemlich kontrahiert, sie haben demnach kein »somatisches» Aussehen. Zuweilen kann man jedoch die Stelle der primären Einschnürung sehen. Drei Chromosomen haben ein submedianes Centromer, zwei Chromosomen ein subterminales, sodass die beiden Schenkel sehr ungleich lang sind, während in zwei Chromosomen der eine Schenkel etwa doppelt so lang ist als der andere (Fig. 2 *e*). Die Längsspaltung der Univalente ist nicht mehr wahrzunehmen, sie wird erst später wieder deutlich. Einigemale wurden abweichende Chromosomen beobachtet. Fig. 1 *c* zeigt eine Zelle, in der ein Univalent beim Centromer zweigeteilt ist. Fig. 3 *d* zeigt eine Zelle mit einem extra Fragment. Sehr häufig liegen die Chromosomen

nicht in der Mitte der PMZ sondern neben der Wand. Solchenfalls wird die Kernspindel später, wenn sie sich stark verlängert, gekrümmt, etwa halbmondförmig, da sie nicht genügend Platz bekommt und wohl auch an den gerundeten Wänden nicht genügend Widerstand findet, als dass die Verlängerung verhindert und die Spindel gerade verbleiben würde. Früher ist hervorgehoben worden, dass die Kernspindel bei schwacher Chromosomenpaarung mit vielen Univalenten sich viel mehr verlängert als wenn Bivalente vorhanden sind (siehe BEADLE, 1933). Die Ausbildung der Kernspindel bei der haploiden *Godetia* ist aber sehr verschieden. Schliesslich sei hervorgehoben, dass ein Univalent zuweilen ausserhalb der Kernspindel liegen kann. Solche Chromosomen scheinen zu degenerieren; sie haben anscheinend kein Vermögen einen Kern zu bilden. Fig. 1 *f* zeigt ein Chromosom ganz neben der Kernspindel (und der sich ausbildenden Kernmembran).

Es ist ziemlich schwierig während der recht langen Zeit, die vor Beginn des Interkinesestadiums verfliesst, auf die Bewegungen der Univalente in der Kernspindel zu schliessen. Nach Auflösung der Kernmembran liegen die Univalente ein wenig zerstreut. In vielen PMZ ordnen sich einige oder alle Univalente zu einer Kernplatte, aber in anderen PMZ findet dies offenbar nicht statt. Fig. 1 *d* zeigt eine PMZ, in der ziemlich unmittelbar nach dem Verschwinden der Kernmembran eine Kernplatte gebildet worden ist, also ebenso schnell wie bei den Diploiden. Dies ist indessen eine Ausnahme, die Regel ist, dass die Univalente sich langsam und nach und nach äquatorial ordnen. In den übrigen in Fig. 1 abgebildeten PMZ ist nach Auflösung der Kernmembran sicher längere Zeit verflossen als in *d*; aber nur in *g* liegen alle Chromosomen in der Kernplatte. Diese ist häufig ungleichmässig; es hat den Anschein, als ob es den Univalenten schwer fiele sich richtig einzustellen.

Häufig kommt es vor, dass sich keines der Chromosomen äquatorial ordnet. In Fig. 2 *a* bilden sie anstatt dessen eine sphärische Gruppe, als ob sie von den Polen keinem Druck ausgesetzt wären; es scheint nur die Repulsion zwischen den Centromeren der Chromosomen zu wirken. In diesem Stadium erscheint dann wiederum die Längsspaltung (Fig. 2 *d*) und es wird ein kugelrunder Interkinesekern gebildet (wie in Fig. 7 *a*). Restitutionskerne von diesem Aussehen waren sehr häufig. Es ist zu beachten, dass die Spaltung nicht das Centromer trifft; dieses verbleibt ungeteilt und hält die beiden Chromatiden zusammen, die auseinander weichen. Chromosomen mit medianem Centromer gleichen dann einem symmetrischen X. Die Chromosomen

können indessen in der PMZ auch zerstreuter liegen. Fig. 2 *b* zeigt ein Beispiel hierfür, obgleich es vielleicht nicht ausgeschlossen ist, dass es in dieser Zelle früher eine Kernplatte gegeben hat. In dieser PMZ wird dann ein grösserer und langgestreckter Interkinesekern gebildet (wie in Fig. 6 *a*).

Ein Univalent ordnet sich also nur mitunter in eine Kernplatte ein.

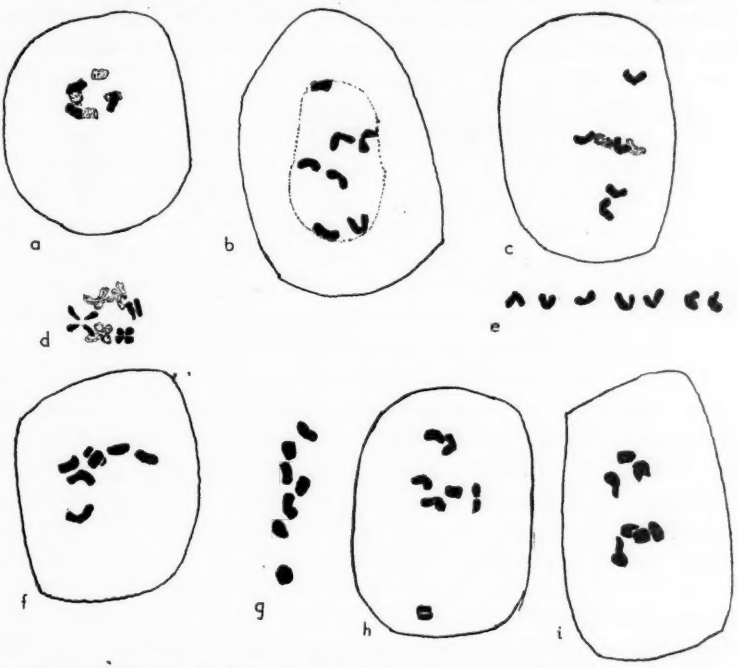


Fig. 2. — *a*: PMZ mit sphärischer Univalentengruppe. — *b*: PMZ mit mehr zerstreuten Univalenten. — *c*: Vier Univalente in der Kernplatte. — *d*: Weiterentwicklung von *a*. — *e*: Die sieben Univalente aus einer Zelle. — *f*: Fünf Univalente in der Kernplatte. — *g*: Univalente auf der Kernspindel zerstreut. — *h*: Drei Univalentengruppen in der PMZ. — *i*: Anaphase mit der Verteilung 3 + 4.

Ist es einmal dahin gekommen, kann es sich indessen auf zwei verschiedene Weisen verhalten: entweder verbleibt es in der Kernplatte oder auch es unternimmt eine Anaphasewanderung nach einem der Pole. Aber, wie sich die Chromosomen auch verhalten mögen, so verbleibt ihr Centromer meistens ungeteilt, also auch wenn es in der Kernplatte liegen bleibt. Zufolge dieser Variation im Verhalten der Univalente entstehen viele verschiedene zytologische Bilder, und nicht wenige von

diesen können nicht mit Sicherheit gedeutet werden. In bezug auf die in Fig. 2 *c* abgebildete PMZ, wo vier Chromosomen eine Kernplatte bilden, lässt sich unmöglich entscheiden, wie sich die ausserhalb der Platte liegenden Univalente verhalten werden. In dem oberhalb der Platte liegenden ist das Centromer gegen die Platte gerichtet, in dem einen der beiden unterhalb der Platte gelegenen Chromosomen ist es von der Platte gerichtet, und im zweiten sind die Chromosomenarme longitudinal orientiert. Die Centromeren würden am ehesten darauf deuten, dass sich alle drei Chromosomen verschieden verhalten, aber es erscheint fast absurd, dass ein Chromosom sich am Weg zur, das zweite von der Platte befindet und das dritte stationär sein sollte; wahrscheinlicher sind alle drei stationär und die Orientierung der Centromeren dürfte eine zufällige sein. Eine andere schwer zu erklärende PMZ zeigt Fig. 2 *f*. Fünf Univalente bilden eine Platte, die nicht äquatorial sondern näher dem einen Pol in der PMZ liegt (die Umgrenzung der Kernspindel war, wie bei Haploiden häufig, undeutlich, wahrscheinlich ist sie oft in irgend einer Weise abnorm), darunter liegen zwei Chromosomen, die ein Bivalent zu bilden scheinen.

Fig. 3 *b* zeigt dagegen unzweideutig eine Polwanderung der Univalente. Hier sind, wie es scheint, alle Centromeren in jeder Gruppe in der Richtung vom Äquator orientiert. Das oberste Chromosom war bei einer anderen Einstellung der Mikrometerschraube mehr sichtbar als die übrigen, weshalb es wahrscheinlich erscheint, dass es niemals in der Äquatorialplatte gelegen hat. Die Befestigung von Zugfasern am Chromosom wurde beobachtet. Fig. 2 *i* zeigt eine 3 + 4 Verteilung; es scheint, als ob es hier ein Bivalent gegeben habe, dessen Chromosomen auf zwei Pole verteilt werden. Bivalente sind in diesem Material so selten, dass es nicht möglich ist zu sagen, ob dies ihr gewöhnliches Verhalten ist. Auch andere Verteilungen wurden beobachtet. Es ist sehr häufig, dass ein Chromosom am einen Pol, sechs beim anderen liegen (eine Verteilung, die bei vielen anderen Haploiden angetroffen worden ist). Häufig beruht dies darauf, dass das einzelne Chromosom von Beginn an dem einen Pol näher lag und bei der Streckung der Spindel diesem näher gebracht worden ist. Es hat aber tatsächlich den Anschein, als ob die Anaphasenverteilung 6 + 1 einer Kernplatte häufiger auftritt, als man auf Grund der Zufallsgesetze erwarten würde. In den Fig. 3 *c* und *d* sehen die Chromosomen der einen Polgruppe ganz so aus wie in einer normalen diploiden Gruppe. Bei der Anaphasenwanderung resultieren zwei Interkinesekerne wenn die beiden Chromosomengruppen gut getrennt sind. Oft sind jedoch die Chromo-

somen längs der Kernspindel zerstreut (Fig. 2 *g*), und dann entsteht ein einziger, aber langer und schmaler Interkinesekern.

Häufig gibt es auf der Kernspindel mehr als zwei Chromosomengruppen. Fig. 2 *h* zeigt am unteren Pol eine, am oberen Pol zwei Chromosomen, während vier Chromosomen zunächst eine unregelmässige Kernplatte bilden. Fig. 3 *a* zeigt ein Chromosom an jedem Pol, während die übrigen nahe des Äquators liegen. Andere Beispiele für drei Gruppen sind Fig. 4 *f* und *g*. Es werden auch, wenngleich selten,

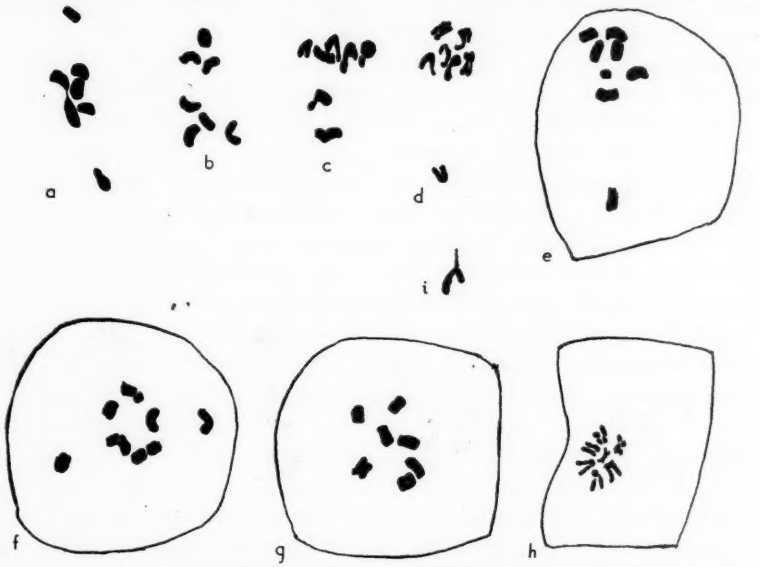


Fig. 3. — *a*: Drei Chromsomengruppen auf der Spindel. — *b*: Anaphase 3 + 4. — *c*: Anaphase 6 + 1. — *d*: Späte Anaphase 6 + 1. — *e*: PMZ mit Fragment. — *f*: PMZ mit Kernplatte und zwei isolierten Chromosomen. — *g*: Quergeschnittene Univalentenplatte. — *h*: Ditto. Weiterentwicklung von *g*. — *i*: Anaphasechromosom mit Zugfasern.

drei Interkinesekerne gebildet (Fig. 6 *d*). Die in Fig. 6 *g* abgebildete Lage der drei Kerne ist vielleicht infolge einer starken Streckung einer peripher liegenden Kernspindel entstanden, wobei ein bzw. zwei Chromosomen, die nahe den Polen gelegen sind, weit weg geschoben worden sind.

Wir kommen schliesslich zu dem Fall, in dem die Univalente sich in eine regelmässige Platte geordnet haben, diese aber dann nicht verlassen. Fig. 3 *f* ist etwas ungewöhnlich. In der gleichen Ebene liegt

eine Kernplatte mit fünf Chromosomen und jederseits dieser ein einzelnes Chromosom. Letztere liegen ausserhalb der Spindel. In Fig. 3 *g* hat die PMZ eine Platte mit 7 Chromosomen, von denen einige eine Andeutung zu Teilung aufweisen; Fig. 3 *h* zeigt auch eine Kernplatte von der Polseite; aber hier haben die Chromosomen begonnen Interkineseform anzunehmen. Fig. 4 *a* zeigt eine solche Kernplatte von der Seite; um eines der Chromosomen gibt es eine Andeutung zu Mem-

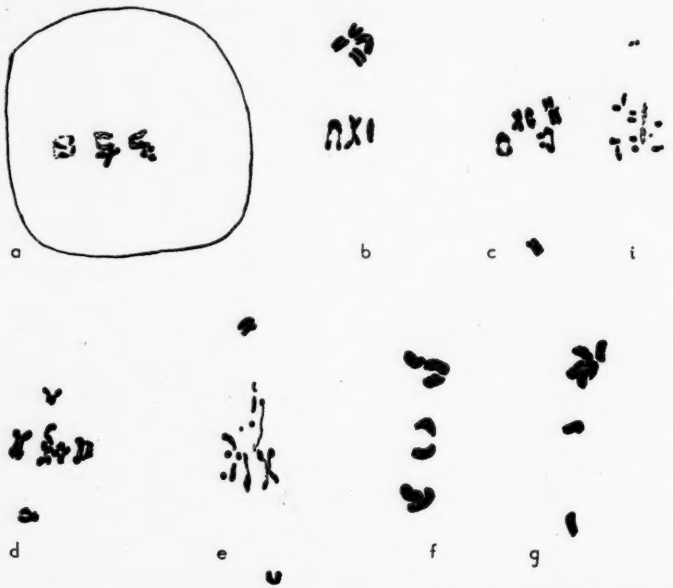


Fig. 4. — *a*: PMZ mit Kernplatte, die Univalente nehmen Interkineseform an. — *b*: Vier Univalente bei dem Pol, drei in der Platte. — *c*: Sechs Univalente in der Platte. — *d*: Fünf Univalente in der Platte. — *e*: Weiterentwicklung aus *a*—*d*, die Plattenunivalente scheinen sehr langgestreckt und teilweise fragmentiert zu sein. — *f* und *g*: Drei Chromosomengruppen auf der Spindel. — *i*: Teilung der Univalente (?).

bran. Die Chromosomen haben auch das gleiche Aussehen wie beim Übergang zu gewöhnlicher Interkinese (vgl. Fig. 5). Oft entsteht grosse Ähnlichkeit mit dem Beginn der Metaphase 2; dass es sich aber nicht um dieses Stadium handelt, ergibt sich bei einem Vergleich mit den anderen PMZ im Kleinfach und aus den Fällen, in denen nur ein Teil der Chromosomen in der Platte liegt. Fig. 4 *b* zeigt vier Chromosomen am einen Pol, während nur drei im Äquator liegen. In Fig. 4 *c* liegen dagegen sechs in der Kernplatte, und in Fig. 4 *d* schliesslich fünf,

während hier ein Chromosom an jedem Pol liegt. Man würde nun erwarten, dass sich die Chromatiden trennten und eine Längsteilung wie bei einer pseudohomotypischen Teilung einträte. Aber ein sicherer solcher Fall ist nicht beobachtet worden. Die Chromosomen in der Platte sehen oft wie kleine Bivalente aus, ihre Chromatiden sind stark ausgespannt, als ob sie von verschiedenen Richtungen einem Zug ausgesetzt seien, aber der Kontakt zwischen ihnen scheint im allgemeinen nicht verloren zu gehen. Es hat den Anschein, als ob die Kernspindel sich verlängert und das Centromer sich am Nullpunkt befindet. Oder es kann aussehen, als ob das Centromer geteilt ist, die Tochtercentromeren aber durch einen Faden verbunden sind. Schliesslich bekommt man mitunter den Eindruck, als ob das Chromosom eine Querteilung statt einer Längsteilung erfahren sollte (»misdivision of centromere»; DARLINGTON, 1939). Die Chromosomenarme sind nämlich oft in der Längsachsel der Spindel (Fig. 2 *h*). Die am Äquator liegenden Chromosomen bekommen hierdurch ein anderes Aussehen als die an den Polen liegenden (Fig. 4 *e*). Die zunächst folgende Entwicklung erschien schlecht fixiert. Sowohl bei 48 wie bei 211 waren die aus der Kernplatte gebildeten Interkinesekerne äusserst undeutlich. Sie waren wenig färbbar, und was man von den Chromosomen sehen konnte waren lange Schleifen oder mehr oder weniger zahlreiche Fragmente (noch undeutlicher als in Fig. 4 *e* abgebildet). Fig. 4 *i* zeigt vielleicht eine Teilung der Univalente.

Bei den *Godetia Whitneyi*-Haploiden kann demnach von einer »zufallsweisen» Verteilung der Univalente auf die beiden Pole keine Rede sein. In den allermeisten PMZ wird ein einziger Interkinesekern mit sieben Chromosomen gebildet. Und wenn zwei Kerne gebildet werden, was in kaum mehr als $^1/_4$ der PMZ vorkommt, so ist es das häufigste, dass der eine nur ein Chromosom enthält (also die »Verteilung 6 + 1»). Aber eine Verteilung von 2 + 5 und 3 + 4 wurde natürlich verschiedenemale beobachtet; sie entsteht am häufigsten durch eine Polwanderung von einer Kernplatte. Wie wenn es sich um die Bivalentbildung handelte, konnte man übrigens auch beim Studium der Meta-Anaphase konstatieren, dass bei den im gleichen Kleinfach gelegenen PMZ eine Tendenz bestand, sich gleichartig zu verhalten. So hatten fast alle PMZ in einem Kleinfach von Pflanze 48 eine Gruppe von sechs Chromosomen und ein einzelnes Chromosom. In einem anderen Kleinfach zeigten dagegen 11 PMZ »7 + 0» und 2 PMZ »6 + 1».

Beim Übergang zur Interkinese ist die Längsspaltung in den Chro-

mosomen wiederum sichtbar, wobei jedoch das Centromer ungeteilt verbleibt. Fig. 3 *c* zeigt eine Anaphase mit noch ungeteilten Chromosomen, die Chromosomenverteilung scheint 6 + 1 zu werden. Fig. 3 *d* hat die gleiche Verteilung in einem etwas späteren Stadium; hier ist die Spaltung deutlich. In Fig. 5 *a* sind die Chromosomen beim Eintritt in das Interkinesestadium längs einer peripheren Kernspindel zerstreut, Fig. 5 *b* ist ähnlich, aber es scheinen zwei Kerne nebeneinander gebildet

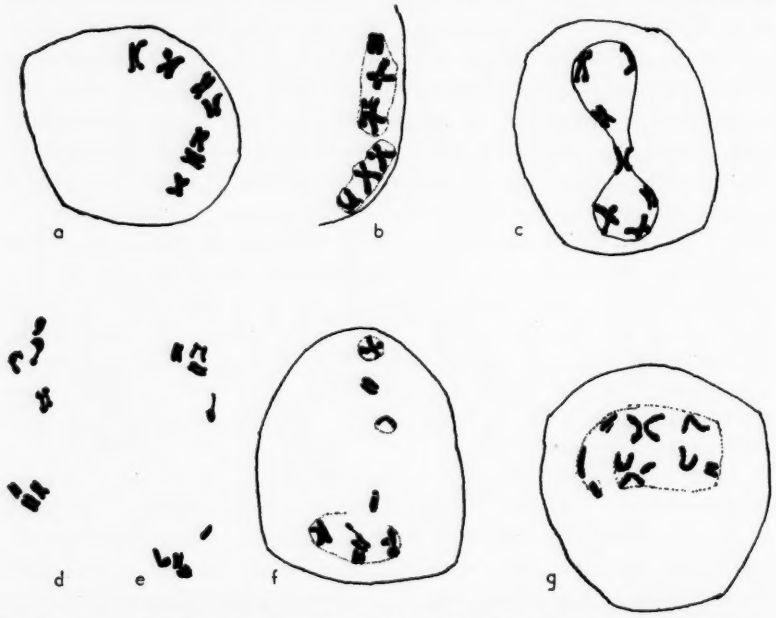


Fig. 5. — *a*—*c*: Übergang zur Interkinese bei verschiedener Lage der Univalenten. — *d*: Anaphase, ein Univalent verspätet. — *e*: Das Centromer in dem verspäteten Univalent ist geteilt. — *f*: Interkinese, ein Centromer ist geteilt. — *g*: Einige Centromere in dem Interkinesekern sind geteilt.

zu werden, die wahrscheinlich bald verschmelzen. Die Kernbildung beginnt rund um jedes Chromosom, und wenn diese getrennt liegen, tritt gelegentlich das Bild von Kleinkernen auf, die dann ineinander übergehen. In Fig. 5 *c* ist ein stundenglasförmiger Interkinesekern gebildet worden.

Zuweilen kommt es wirklich zu einer Teilung des Centromers. Dies kann in einem in der Kernspindel verzögerten Chromosom vorkommen (was auch bei untersuchten di-polyploiden Typen beobachtet

worden ist). Fig. 5 *d* zeigt drei Chromosomen an jedem Pol, während ein verspätetes Univalent sich in Teilung befindet. Fig. 5 *e* ist ähnlich, aber die beiden Längshälften sind weit getrennt. Fig. 5 *f* zeigt eine unregelmässigere Verteilung. Am oberen Pol liegen zwei Chromosomen, von denen das eine sich zu einem Kleinkern ausgebildet hat; am unteren Pol gehören vier Chromosomen zu einem Kern, dessen Membran noch nicht fertig ist. In der Mitte der PMZ gibt es zwei Chromatiden, deren Umbiegung zeigt, dass das Centromer in einem Univalent geteilt worden ist, und dass darauf eine Repulsion der Tochtercentromeren stattgefunden hat. Das obere dieser Chromatiden hat einen eigenen Kleinkern gebildet, in bezug auf das untere ist es ungewiss, ob es in den grossen Kern eingehen wird. Fig. 5 *g* zeigt einen Interkinesekern, in dem die Centromeren in einigen der Chromosomen geteilt sind; am deutlichsten in bezug auf die beiden V-förmigen Chromatiden rechts im Kern.

Interkinese. — Im Interkinesekern wurden einige Beobachtungen über Nukleolen gemacht. Seit HEITZ ist nachgewiesen, dass Nukleolen an bestimmten Chromosomen, so besonders an SAT-Chromosomen, gebildet werden.' 'In den Fällen wo SAT-Chromosomen fehlen, gleichwie auch achromatische Einschnürungen an anderen Chromosomen, werden Nukleolen an den Enden gewisser Chromosomen gebildet (siehe GEITLER, 1938, S. 29 und MATSUURA, 1939). Da ich keine guten somatischen Teilungen zu studieren Gelegenheit hatte, kann ich mich über das ev. Vorkommen von achromatischen Teilen in den *Godetia*-Chromosomen nicht äussern; aber wahrscheinlich gibt es SAT-Chromosomen. Es zeigt sich nämlich, dass in der Interkinese zwei Chromosomen »nukleolenbildend» sind. Das eine dieser Chromosomen hat ein subterminales Centromer und einen Nukleolus am einen Ende, wo sich wahrscheinlich ein Satellit befindet, wenngleich er in diesem Stadium undeutlich zutagetritt (siehe Fig. 6 *b* u. *f*). Das zweite hat den Nukleolus submedian (siehe Fig. 6 *e*).

Das Chromosom, das den Nukleolus terminal bildet, bekommt eigentümlicherweise gern Zwillingsnukleolen von ganz gleicher Grösse (Fig. 6 *e* und *g*). Diese scheinen später zu einem vereinigt worden zu sein (Fig. 7 *a*). Es ist schon hervorgehoben worden, dass die Chromosomen in diesem Stadium längsgespalten sind; das Vorkommen von Zwillingsnukleolen zeigt, dass der nukleolenbildende Teil des Chromosoms auch doppelt ist. Die Grösse des Nukleolus ist vom Chromosom abhängig; es zeigt sich ja, dass zwei identische nukleolenbildende Teile auch gleich grosse Nukleolen bilden.

Wenn die PMZ einen Interkinesekern enthält, hat er in den meisten Fällen nur einen einzigen Nukleolus, und dieser pflegt an dem Chromosom befestigt zu sein, das den Nukleolus terminal ausbildet; dieses ist also am wichtigsten, es hat das grösste nukleolenbildende Vermögen (Fig. 6 *g*, 7 *a*, während Fig. 6 *a*, wie es scheint, ein anderes Verhalten aufweist). Seltener bilden beide Chromosomen im Kern einen Nukleolus (Fig. 6 *e*). Fig. 6 *b* zeigt eine Interkinese mit der

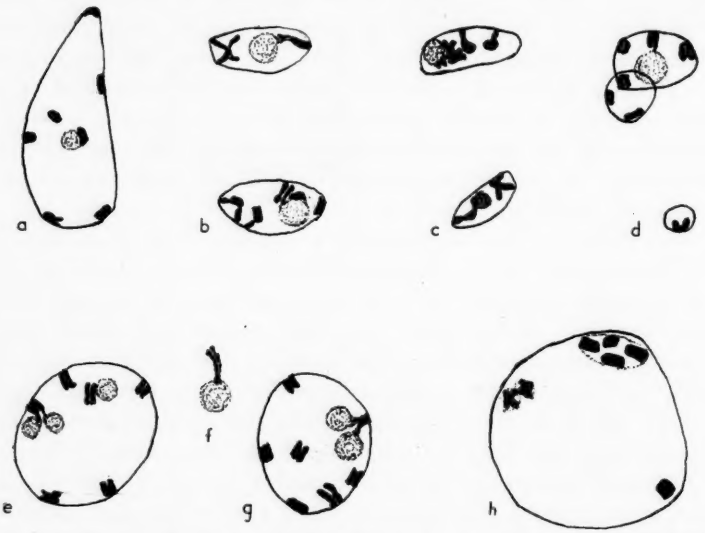


Fig. 6. — *a*: Interkinesekern mit einem Nukleolus. — *b* und *c*: die »nukleolenbildenden» Chromosomen sind auf zwei Kerne verteilt. — *d*: Sie sind wahrscheinlich beide in dem Interkinesekern mit vier Chromosomen. — *e*: Beide haben Nukleolen gebildet, das »terminale» Zwillingsnukleolen, trotzdem sie sich in demselben Kerne befanden. — *f*: Das »terminale» Nukleolenchromosom. — *g*: Kern mit Zwillingsnukleolen. — *h*: PMZ mit drei Interkinesekernen.

Chromosomenverteilung 5 + 2 und die beiden nukleolenbildenden Chromosomen liegen in verschiedenen Kernen. Ähnlich ist Fig. 6 *c*. Die Abwesenheit einer Konkurrenz vom »terminalen Chromosom» gibt dem anderen Gelegenheit einen Nukleolus auszubilden. In Fig. 8 *a* haben dagegen die beiden Pollenzellen mit zwei Chromosomen keinen Nukleolus, und dies dürfte wohl darauf beruhen, dass beide nukleolenbildende Chromosomen in den 5-chromosomigen Interkinesekern eingegangen sind. Von den drei Interkinesekernen in Fig. 6 *d* enthält nur einer, der mit vier Chromosomen, einen Nukleolus, was wahrschein-

lich auf das Gleiche beruht, dass nämlich beide Nukleolenchromosomen in den gleichen Kern gelangt sind.

MATSUURA (1939) hat gezeigt, dass es bei *Trillium kamtschaticum* zwei nukleolenbildende Chromosomen gibt (die Nukleolen werden am einen Ende von Chromosom *A* und *E* gebildet). Bei asynaptischen

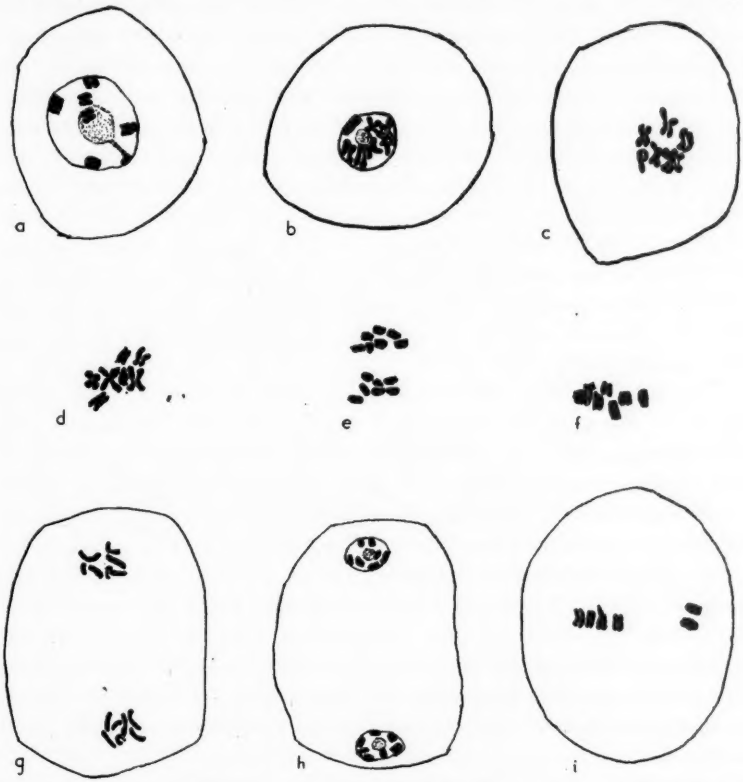


Fig. 7. Die zweite Teilung. — *a* und *b*: Interkinese. — *c* und *d*: Übergang zur Metaphase 2. — *e*: Anaphase 2. — *f*: Metaphase 2. — *g*: Späte Anaphase. — *h*: Die Pollenkerne sind gebildet. — *i*: PMZ mit zwei Kernspindeln.

Pflanzen, wo die Chromosomen in der Meiosis isoliert bleiben und verschiedene Kleinkerne bilden, zeigte es sich, dass alle das Vermögen zur Bildung eines Nukleolus haben. Dieses Vermögen ist bei *A* und *E* nur viel grösser, sodass die Anwesenheit eines dieser in einem Kern die anderen Chromosomen an der Bildung eines Nukleolus hindert. Aber

es scheint, als ob haploide *Godetia* sich in anderer Weise verhielten. Man findet häufig Kerne mit einem oder einigen wenigen Chromosomen ohne deutlichen Nukleolus. Alle Chromosomen scheinen hier kaum das Vermögen zur Bildung von Nukleolen zu besitzen.

Die zweite Teilung. — Diese wurde nicht so eingehend studiert, wie erwünscht war, da sie recht schlecht fixiert war. Fig. 7 *a*—*h* zeigt den Verlauf der Teilung eines Interkinesekerns mit 7 Chromosomen. Er erfährt zuerst gern eine Kontraktion (Fig. 7 *b*), Kernmembran und Nukleolus werden aufgelöst, und die Chromosomen, deren Chromatiden stark auseinander weichen, aber von den Centromeren zusammengehalten werden, liegen zuerst ein wenig unregelmässig (Fig. 7 *c* und *d*), ordnen sich aber dann zu einer Kernplatte (Fig. 7 *f*). Wenn die Metaphase ihren Höhepunkt erreicht, sind die Chromosomen stärker kontrahiert und nicht mehr X-ähnlich (Fig. 7 *f*, vgl. 7 *i*). Die Anaphase ist in Fig. 7 *e* und *g* abgebildet, die Schwesterchromatiden trennen sich. In den 7-chromosomigen Telophasenkernen wird der Nukleolus am einen Chromosom gebildet (Fig. 7 *h*). Schliesslich wird eine Pollendiade gebildet (Fig. 8 *a*).

Wenn es mehrere Interkinesekerne gibt, werden sie alle geteilt (Fig. 7 *i*). Sie konnten nicht näher studiert werden. Wenn der eine Interkinesekern nur ein Chromosom hatte, wurde seine Membran jedoch aufgelöst und er teilte sich; aber es hatte den Anschein, als ob er während dieser Teilung zuweilen in die grössere Kernteilungsfigur einverleibt wurde. Solchenfalls sollte aus zwei Interkinesekernen mit 6 und 1 Chromosomen anstatt einer Tetrade mit zwei Zwergzellen eine Diade entstehen. Nicht selten wurde nur eine Zwergzelle beobachtet. Wahrscheinlich haben sich die Chromatiden nicht getrennt. Zurückgebliebene Chromosomen wurden während der zweiten Teilung beobachtet. Es handelt sich, wie aus anderem *Godetia*-Material hervorgeht, um Chromatiden von Chromosomen, deren Centromeren früher geteilt worden sind.

Sporaden. — Als Folge der Meiosis resultiert eine grosse Anzahl von Pollendiaden, obgleich es offenbar ist, dass der % in verschiedenen Kleinfächern beträchtlich variiert. Ferner gibt es Tetraden mit verschieden grossen Zellen (siehe Fig. 8 *a*), Triaden, deren Bildungsweise nicht klargelegt werden konnte, was auch für vereinzelte Monaden gilt. Auch Pentaden wurden, wenngleich selten, beobachtet (siehe Fig. 8 *b*). Bei einer Auszählung waren von 182 Sporaden 129 Diaden, also 71 %. Ein grosser Teil des Pollens bekam also trotz der Haploidie die normale Chromosomenzahl. Ferner gab es 28 Tetraden, also 15 %, 11

Triaden und 2 Pentaden. Trotz des Verlaufes der Meiosis ist diese Haploide indessen nur schwach fertil. Bei Selbstung von 48 wurden keine Nachkommen erhalten. Dass aber befruchtungsfähige Pollenkörner gebildet wurden, zeigte deutlich diploides ♀ × haploides ♂, welche Kreuzung als Resultat 28 Pflanzen lieferte.

Schliesslich sei erwähnt, dass bei der haploiden Pflanze von *Godetia amoena* die Meiosis in anderer Weise verlaufen ist als bei *Whitneyi*. Teilungsstadien gab es im fixierten Material nicht, aber die Frequenz von Diaden unter den Sporaden war, auf Grund der recht fragmentarischen Studien zu urteilen, niedrig. So wurden in einer Anthere, die teilweise Sporaden enthielt, 4 Diaden, 4 Triaden, 8 Tetraden, 6 Pentaden und 6 Hexaden gezählt. Es ist klar, dass hier die Univalente mehr

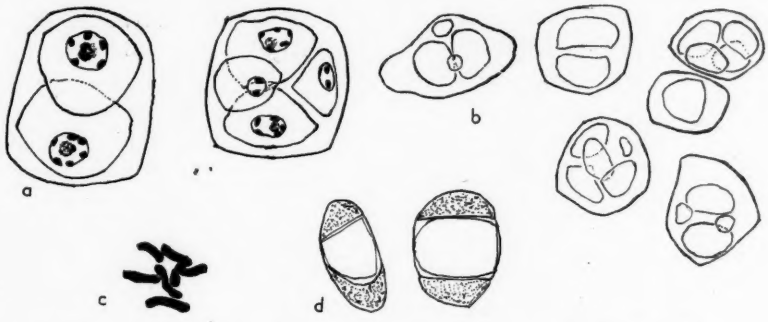


Fig. 8. — *a* und *b*: Sporaden. — *c*: *G. amoena*. Somatische Kernplatte. — *d*: *G. amoena*. Pollenkörner.

zerstreut gewesen sind, sodass nur selten ein, dagegen oft drei Interkinesekerne gebildet worden sind. Die Chromosomenzahl wurde in somatischen Platten bestimmt. Fig. 8 *c* ist von einer Tapetumzelle. Diese pflegen in der Regel viele Chromosomen zu enthalten, aber mitunter bleibt die Chromosomenvermehrung aus.

Die Pollenkörner der haploiden *amoena* waren von sehr variierender Grösse und Form. Zwei sind in Fig. 8 *d* abgebildet. Indessen scheint Nr. 214 laut Angaben von Dozent HIORTH fertiler zu sein als haploide *Whitneyi*. Bei Selbstung wurde eine geringe Anzahl Samen erhalten, bei spontaner Kreuzung eine viel grössere Anzahl. Die Eizellen sind hier vielleicht besser als bei haploider *Whitneyi*.

Die ausserordentlich hohe Prozentzahl von unreduzierten Pollenkörnern ist überraschend. Pollendiaden sind bei vielen Haploiden fest-

gestellt worden, und auch dass solche aus unreduzierten Zellen bestehen können, aber der Prozent PMZ mit ausgebliebener Reduktion ist gering und schwankend, da er von äusseren Faktoren beeinflusst zu werden scheint. Ein recht hoher % wurde bei haploider *Datura stramonium* gefunden; nach Auszählung von Diaden wurden in einem Fall 10 %, aber in einem anderen 29 % Non-Reduktion berechnet (BELLING und BLAKESLEE, 1927); in einer diploiden Form, die durch ein rezessives Gen bedingte vollständige Asynapsis hatte, gab es dagegen nur 0—8 % Non-Reduktion (BERGNER, CARTLEDGE und BLAKESLEE, 1934). Nur bei einer haploiden *Matthiola incana* mit 7 Chromosomen und einem Fragment wurde eine ähnliche Prozentzahl wie bei *Godetia*, nämlich 70, gefunden, obgleich hier der Mechanismus, der zur Bildung der Diaden führte, ein anderer war (LESLEY und FROST, 1928).

Bei haploider *Whitneyi* wird das Centromer der Univalente während der Meiosis einmal geteilt, und dies ziemlich spät, sodass es bei Eintritt der Interkinese noch nicht stattgefunden hat. Nur selten erfolgt die Teilung früher und dann in einem verspäteten Anaphasechromosom, dessen Chromatiden dann häufig Zwergkerne bilden; diese können aber auch in einen grösseren Interkinesekern aufgehen. In der Regel liegen die Univalente so nahe aneinander, dass nur ein Interkinesekern gebildet wird. Dies beruht darauf, dass sich die Kernspindel nicht nennenswert gestreckt hat oder dass die Chromosomen bei der Streckung sehr zerstreut liegen. Eine variierende Anzahl von Univalenten bildet eventuell eine Kernplatte, sie sind, wie man sich auszudrücken pflegt, bipolar geworden, sie werden von den beiden Polen in der Kernspindel repelliert. Dieser bipolare Zustand geht bisweilen nicht verloren, sondern der Interkinesekern wird um die Äquatorchromosomen gebildet, die hierbei gern ein eigentümliches Aussehen annehmen (teilweise ähnlich dem von Pflanze B 7 von asynaptischem *Pisum sativum*; siehe KOLLER, 1938). In der Regel werden auch in diesem Fall die Centromeren nicht geteilt. Dagegen ist offenbar, dass die Univalente die Kernplatte verlassen und nach einem der Pole wandern können. Überhaupt verhalten sie sich wie eventuell vorkommende Univalente von diploiden *G. Whitneyi*-Formen (siehe HÅKANSSON, 1940).

Wenn haploide Pflanzen unreduzierte Pollenkörner bilden, kann dies, wie bei *G. Whitneyi*, durch die Bildung von Restitutionskernen geschehen, indem die Centromeren der Chromosomen bis zur zweiten Teilung ungeteilt verbleiben. Es resultiert eine Pollendiade. In anderen Fällen kommt es zu einer (laut GUSTAFSSON, 1935) pseudohomotypischen Teilung. Die Univalente bilden eine Kernplatte, ihre Centro-

meren teilen sich in dieser und es werden zwei Tochterkerne gebildet. Eine neue Teilung findet nicht statt, sondern auch hier resultiert eine Pollendiade.

Der erste Weg ist wohl der häufigste. Bei haploidem *Triticum durum coerulescens* können wie bei *Godetia* ein oder mehrere Interkinesekerne gebildet werden, abhängig von der Lage der Chromosomen in der Kernspindel (KIHARA, 1936). Laut KIHARA liegen bei *Triticum*-Bastarden eventuelle Univalente in früher Metaphase im allgemeinen an den Polen, um darauf hinunter in die Äquatorialregion zu wandern und eine Kernplatte zu bilden. Bivalente ordnen sich dagegen früh in die Platte ein. Wenn nun bei in Frage stehenden Haploiden die Univalente in die Interkinese übergehen, während sie sich an den Polen befinden, so werden zwei Interkinesekerne (eine Regressionsdiade) gebildet. Erfolgt dies später, wenn alle oder ein Teil von ihnen den Äquator erreicht haben, so wird ein Kern (eine Regressionsmonade) von sehr variierender Form gebildet. Die Entstehung einer Regressionsdiade als Folge von Anaphasenwanderung der Univalente von der Platte nach den Polen kommt also hier nicht vor, dagegen aber sicherlich bei der haploiden *G. Whitneyi*. Ein Restitutionskern wird zuweilen bei haploiden *Oenothera blandina* gebildet (CATCHESIDE, 1932). Hier sollen sich die Univalente früh in eine Kernplatte einordnen. Später erfolgt die Polwanderung der Univalente, und wenn diese sehr zerstreut liegen, kann ein einziger Interkinesekern gebildet werden.

Laut BLEIER (1933) ordnen sich dagegen bei haploiden *Oe. franciscana* und *Hookeri* die Univalente erst spät in eine Kernplatte ein. Die Bildung von zwei Kernen erfolgt also bei diesen wie bei *Triticum* und nicht wie bei *Oe. blandina*. Die Univalente, die die Kernplatte erreicht haben, erleiden eine Längsteilung und die »Spalthälften» werden entweder auf zwei Pole verteilt oder sie gelangen in einen einzigen Kern. Es geht nicht richtig klar hervor, ob eine Teilung der Centromeren immer stattgefunden hat, aber es erscheint wahrscheinlich, dass hier der Restitutionskern von der Kernplatte gebildet werden kann. BLEIER ist ferner der Ansicht, dass die Chromosomen in beiden Teilungen geteilt werden können, dass also die Centromeren zweimal geteilt werden, aber andere Forscher haben dies nicht gefunden. Bei diesen beiden Haploiden sollten demnach laut BLEIER beide Typen und überdies doppelte Teilungen der Centromeren vorkommen können. Bei haploider *G. Whitneyi* kann vielleicht zuweilen auch eine pseudohomotypische Teilung vorkommen, was jedoch nicht sicher festgestellt worden ist; aber die Erfahrungen von anderen *Godetia*-Formen zeigen, dass das

Centromer nur einmal geteilt wird (HÅKANSSON, 1940), nach der pseudohomotypischen Teilung also eine Pollendiade gebildet wird.

Ein typisches Beispiel für pseudohomotypische Teilung haben wir bei der früher erwähnten haploiden *Matthiola incana*. Hier bilden die Univalente eine Kernplatte, erfahren eine Längsteilung, worauf zwei Tochtergruppen gebildet werden. Die zweite Teilung unterbleibt und es entsteht eine Pollendiade. Nur in einer geringeren Anzahl von PMZ haben sich die Chromosomen nicht geteilt, sondern wurden nach dem Zufall auf die zwei Pole verteilt. In diesen PMZ fand dann die zweite Teilung statt und es wurden Triaden und Tetraden gebildet. Dies scheint das beste Beispiel für eine pseudohomotypische Teilung bei Haploiden zu sein. Aus den Beschreibungen von haploiden *Datura stramonium* könnte man zur Auffassung gelangen, dass diese Teilung hier stattfindet, aber eine spätere Abhandlung über eine asynaptische diploide *Datura* gibt an, dass die Univalente sich dort wie bei den haploiden verhalten und diese diploide Form bildet Restitutionskerne. Bei haploiden *Crepis capillaris* sollen die Univalente in seltenen Fällen in der Anaphase 1 geteilt werden (HOLLINGSHEAD, 1930). Einwände gegen die Deutung der Stadien in dieser Arbeit sind jedoch vorgebracht worden (BLEIER, l. c. S. 157).

SUMMARY.

Meiosis in two haploid plants of *Godetia Whitneyi* was studied.

Bivalents occur in less than 1 % of the pmc. The bivalents have a terminal or a subterminal chiasma. A few pmc had a ring bivalent. The bivalents indicate duplications in the haploid chromosome set.

In many pmc no metaphase plate is formed, in other pmc all or some of the univalents form a plate.

The univalents in the plate may go to the poles or remain in the plate.

Only very seldom does a centromere divide before interkinesis.

In most pmc only one interkinesis nucleus is formed. If there are two or three well separated groups of chromosomes two or three interkinesis nuclei are formed.

In interkinesis nuclei two chromosomes are able to form a nucleolus. One has the nucleolus terminally and may form twin nucleoli, the other has the nucleolus submedially. The former is the more important, because when both chromosomes are in the same nucleus, in most cases only this one has a nucleolus.

The second division proceeds normally, the centromeres splitting. About 70 % pollen diads with the unreduced chromosome number are formed.

A haploid *G. amoena* had few diads but pentads and hexads.

ZITIERTE LITERATUR.

1. Beadle, G. W. 1933. Further studies of asynaptic maize. — Cytologia 4.
2. Belling, J. and Blakeslee, A. F. 1927. The assortment of chromosomes in haploid *Daturas*. — La Cellule 37.
3. Bergner, A. D., Cartledge, J. L. and Blakeslee, A. F. 1934. Chromosome behaviour due to a gene which prevents metaphase pairing in *Datura*. — Cytologia 6.
4. Bleier, H. 1933. Die Meiosis von Haplobionten (*Oenothera franciscana* und *Oe. Hookeri*). — Genetica 15.
5. Catcheside, D. G. 1932. The chromosomes of a new haploid *Oenothera*. — Cytologia 4.
6. Darlington, C. D. 1939. Misdivision and the genetics of the centromere. — Journ. of Genetics 37.
7. Emerson, S. H. 1929. The reduction division in a haploid *Oenothera*. — La Cellule 39.
8. Geitler, L. 1938. Chromosomenbau. — Protoplasma-Monographien, Bd. 14.
9. Gustafsson, Å. 1935. Studies on the mechanism of parthenogenesis. — Hereditas XXI.
10. Hollingshead, L. 1930. A cytological study of haploid *Crepis capillaris*. — Univ. Cal. Pub. Agr. Sci. 6.
11. Håkansson, A. 1940. Die Meiosis bei verschiedenen Mutanten von *Godetia Whitneyi*. — Lunds Univ. Årsskrift. N. F. Avd. 2, Bd. 36, Nr. 5.
12. Ivanov, M. A. 1938. Experimental production of haploids in *Nicotiana rustica*. — Genetica 20.
13. Kihara, H. 1936. Ein diplo-haploides Zwillingpaar bei *Triticum durum*. — Agriculture and Horticulture 11.
14. Koller, P. C. 1938. Asynapsis in *Pisum sativum*. — Journ. of Genetics 36.
15. Lesley, M. M. and Frost, H. B. 1928. Two extreme »small» *Matthiola*-plants: a haploid with one and a diploid with two additional chromosome fragments. — Amer. Nat. 62.
16. Matsuura, H. 1939. Chromosome studies on *Trillium kamtschaticum*. VI. On the nucleolus-chromosome relationship. — Cytologia 9.

CHROMOSOME NUMBERS AND POLYPLOIDY WITHIN THE FLORA OF SPITZBERGEN

BY *KARL FLOVIK*

STATE AGRICULTURAL EXPERIMENT STATION, HOLT, TROMSÖ, NORWAY

THE extensive investigations of recent years on both experimentally produced polyploids and polyploids in nature have led to results which have greatly increased our knowledge of the evolutionary methods of nature. There can hardly be any doubt at present that polyploidy (the term including both autopolyploidy and allopolyploidy) has played a very prominent evolutionary role.

One of the problems to which many investigators have devoted their attention is the adaption of polyploids to extreme conditions of life. In this connexion it is of interest to call attention to the fact that in those cases in which both diploid and polyploid »races» occur within a Linnean species it has frequently been found that the polyploids have a more northern or alpine habitat than the diploids. As a classic example of this we may mention *Biscutella laevigata* (MANTON, 1934). The polyploid forms of this species have their habitat in Central Europe, within those regions which were ice-covered during the great glaciation, while the older, diploid forms have their habitat in the lower lying regions that were not formerly covered with ice. In *Empetrum* we have an example where the polyploid race, *E. hermaphroditum*, has a pronouncedly arctic distribution, while the diploid *E. nigrum* has a more southern habitat (HAGERUP, 1927). In this case the polyploid race has been made into a separate species. Several instances of this kind have been recorded by various authors (cf. MÜNTZING, 1936).

As known, the above-mentioned state of things, however, is not by any means an absolute rule, many cases in which conditions are the reverse having been met with. As extremes in this respect may be mentioned *Vaccinium uliginosum* (HAGERUP, 1933) and *Campanula rotundifolia* (BÖCHER, 1936). In these two species the diploids have a more marked arctic distribution than the polyploid races.

Apart from such intraspecific chromosomal races as those mentioned above, several authors have called attention to the fact that species

with high chromosome numbers seem to have a more northernly or alpine habitat than closely related species, within the same genus or family, with lower chromosome numbers. TÄCKHOLM (1922) first demonstrated this difference in geographical distribution between species with different chromosome numbers in the genus *Rosa*, species with the most northern area of distribution having the highest number, while species with lower numbers are more confined to a warmer climate. With regard to the *Bicornes* group HAGERUP (1928) was able to show that the species growing farthest to the north were invariably those with the highest polyploid numbers. Similar conditions seem to prevail within the *Cactaceae* (cf. STOCKWELL, 1935).

This interesting correlation between polyploidy and geographical distribution has been extensively studied by HAGERUP (1928, 1931) and especially by TISCHLER (1934). These investigators have emphasized the fact that polyploidy is a very important means, which in many cases renders possible an advance towards and an occupation of climatically unfavourable regions in which the diploids do not thrive. In other words the polyploids seem to be better adapted for extreme environmental conditions than the diploids. Consequently, the flora in such unfavourable regions should be richer in polyploids than usual, and TISCHLER has shown this to be the case in a comparison between the flora of Sicily and Iceland. While only 31 per cent of the examined species from Sicily were polyploids, the corresponding figure for Iceland was 55 per cent. Illustrative examples of this tendency to an increased number of polyploid elements in the flora from climatically unfavourable regions have been recorded recently by SOKOLOVSKAJA and STRELKOVA (1938). These authors have made extensive cytological studies of the flora of the high-alpine regions of Pamir and Altai. In Pamir, where very unfavourable conditions, climatic as well as all other growth requirements, prevail, polyploids were predominant, viz. 85 per cent polyploids among altogether 150 species examined. From the high-alpine zone of Altai, where the climate is somewhat milder than in Pamir, 200 species were examined, and of these 65 per cent were found to be polyploids.

Although an extensive material is already available to throw light on various problems in connexion with polyploidy, opinions still differ not the least as to the relation between polyploidy and geographical distribution. In connexion with the above-mentioned observations the present investigation of the high-arctic flora from Spitzbergen may therefore be of interest.

The material on which this investigation is based was collected at Isfjorden. The great majority of the chromosome numbers was determined in root-tip preparations. The fixative employed was LA COUR's 2 BD or other fixatives containing osmic acid. A few sections were fixed in »Navashin». The stain used was gentian violet.

Some of the chromosome numbers found in material from Spitzbergen have already been published, viz. those for the genus *Ranunculus* and the family *Gramineae* (FLOVIK, 1936, 1938), but in a complete survey like this it may be of interest to include also those in the list. The species examined and their chromosome numbers are tabulated in the following list — the diploids are denoted by an asterisk (*).

Species	2n
Gramineae.	
Alopecurus alpinus SM.	112 + 3ff, 114 + 2ff, about 130 + 1f
Arctagrostis latifolia (R. BR.) GRISEB.	62
Arctophila fulva (TRIN.) RUPR.	42
Calamagrostis neglecta (EHRH.) P. B.	28
Deschampsia alpina ROEM. et SCHULT.	39, 41, 49
Dupontia Fisheri R. BR.	88 + ff
Dupontia Fisheri R. BR. var. *psilosantha* (RUPR.) SCHOL.	44 + ff
Festuca rubra L. var. *arenaria* (OSB.) E. FRIES	42 (+ 1f)
Festuca ovina L. var. *brevifolia* (R. BR.) HART	28
Festuca ovina L. var. *vivipara* L.	49
Hierochloe alpina (LILJEBL.) ROEM. et SCHULT.	56
Phippsia algida (SOLAND.) R. BR.	28
Phippsia concinna (TH. FRIES) LINDEB.	28
Phippsia concinna (TH. FRIES) LINDEB. var. *algidiformis* (SMITH) HOLM.	29
Poa abbreviata R. BR.	76 ±
Poa alpigena (E. FRIES) LINDM.	84, 77 ±
Poa alpigena (E. FRIES) LINDM. var. *colpodea* (TH. FRIES) SCHOL.	51 + 5ff
Poa alpigena (E. FRIES) LINDM. var. *vivipara* (MALMGR.) SCHOL.	42 + 4ff
Poa glauca VAHL.	70—72
Poa alpina L. var. *vivipara* L.	44, 42 + 4ff
Poa arctica R. BR.	56
Poa arctica R. BR. var. *vivipara* (MALMGR.) SCHOL.	56
Puccinellia angustata (R. BR.) RAND. et REDF.	42

Puccinellia phryganodes (TRIN.) SCRIBN. et MERR. 28
Puccinellia Vahliana (LIEBM.) SCRIBN. et MERR. 14*
Trisetum spicatum (L.) RICHT. 28

Betulaceae.

Betula nana L. 28

Polygonaceae.

Oxyria digyna (L.) HILL. 14*
Polygonum viviparum L. ..... about 100

Caryophyllaceae.

Honckenya peploides (L.) EHRH. 66
Stellaria longipes GOLDIE 104
Stellaria humifusa ROTTB. 26*
Cerastium Regelii OSTENF. 72
Silene acaulis L. 24*

Ranunculaceae.

Ranunculus Pallasii SCHLECHT. 32
Ranunculus lapponicus L. 16*
Ranunculus Pallasii × *R. lapponicus* 24
Ranunculus hyperboreus ROTTB. 32
Ranunculus pygmaeus WAHLENB. 16*
Ranunculus nivalis L. 48
Ranunculus sulphureus SOLAND. 96

Papaveraceae.

Papaver radicatum ROTTB. 70

Cruciferae.

Cardamine pratensis L. 64
Draba alpina L. 80
Draba rupestris R. BR. 48
Draba arctica WAHLENB. 80
Cochlearia officinalis L. var. *groenlandica* (L.) GELERT. .. 14*

Saxifragaceae.

Saxifraga stellaris L. var. *comosa* RETZ. 56
Saxifraga nivalis L. 60
Saxifraga nivalis L. var. *tenuis* WAHLENB. 20*
Saxifraga hieraciifolia WALDST. et KIT. 112
Saxifraga oppositifolia L. 52

Saxifraga flagellaris WILLD.	32
Saxifraga hirculus L.	32
Saxifraga rivularis L.	26*
Saxifraga groenlandica L.	80
Chrysosplenium alternifolium L. var. *tetrandum* LUND.	24*
Rosaceae.	
Potentilla pulchella R. BR.	28
Potentilla emarginata PURSH.	42
Dryas octopetala L.	18*
Empetraceae.	
Empetrum hermaphroditum (LANGE) HAGERUP	52
Ericaceae.	
Vaccinium uliginosum L.	24*
Polemoniaceae.	
Polemonium boreale ADAMS	18*
Campanulaceae.	
Campanula rotundifolia L.	34*
Compositae.	
Erigeron uniflorus L. var. *unalaschkensis* (D. C.) OSTENF.	36
Petasites frigidus L.	60
Arnica alpina LAEST.	56
Taraxacum arcticum DAHLST.	40

Owing to the existing uncertainty as to the origin of the different chromosome numbers in the *Cyperaceae*, the numbers found in representatives of this family have not been included in the above list in so far as the recording of the polyploid elements is concerned. Of representatives of other families of higher plants the investigation comprises 68 species and varieties, 54 of which are polyploids and 14 diploids, i. e. about 80 per cent polyploids.

This high percentage of polyploids among the Spitzbergen flora agrees very well with the investigations of chromosome numbers in plants from other regions with extreme climatic or otherwise unfavourable conditions of growth, referred to above. The facts mentioned above may be interpreted as an evident tendency indicating that an increased chromosome number increases the adaptability to unfavourable habitats.

It is a well-known fact that an increase in the number of chromo-

somes frequently brings about considerable physiological and other changes in character (cf. MÜNTZING, 1936). — HAGERUP (1931, 1933) has pointed out in his works that with polyploidy there may follow a change in the ecological and plant-geographical value of the plant. Owing to its immediate revolution an increase in chromosome number may therefore be conceived to create at one stroke new forms that are more suited than the diploids to immigrate into and colonize new regions. But perhaps the greatest significance of an increase in the number of chromosomes lies in the fact that *it creates a foundation for a wider range of variation, which in turn leads to greater possibilities for the selection of types capable of colonizing regions where the adaptability of the diploids is inadequate to do so.* As a natural consequence of this increased range of variation the polyploids thus have as a rule a more extensive distribution than the diploids. But even to this rule there are exceptions. Owing to the gigantism that polyploidy frequently brings about, the possibilities of distribution may decrease. On the other hand, though many diploids are only of local importance, others are spread over large areas, partly with highly varying conditions of life, such as was shown by TÜRESSON (1938).

Among the examined species from Spitzbergen there are several that are worth while mentioning more in detail. This is especially true of the examples of intraspecific chromosome races met with. Of these, *Dupontia Fisheri* and *D. Fisheri* var. *psilosantha* have already been reported in detail (FLOVIK, 1938). Although both forms are typically arctic, the high chromosomal *D. Fisheri* seems to extend farthest to the north and otherwise occupies more unfavourable regions than var. *psilosantha*, from which it may have originated by a simple chromosome doubling.

In *Honckenya peploides* ROHWEDER (1936) found the chromosome numbers $2n = 48$ and 64. In this species from Spitzbergen a divergent, and at the same time the highest, number, $2n = 66$, was found. This aneuploid number does not seem to be a chance occurrence, for it was also established for certain in material of *H. peploides* from Tromsö (arctic Norway). The obviously constant occurrence of this aneuploid number is possibly due to apomixis, which appears to be quite common in arctic plants.

In material of *Cardamine pratensis* from Cambridge MANTON (1932) found two different chromosome numbers, viz. $2n = 32$ and 64. In Spitzbergen only the 64-chromosome race was found, i. e. the one with the higher multiple.

The tetraploid *Empetrum hermaphroditum*, which is the sole prevailing form in East Greenland (HAGERUP, 1927), occurs also in Spitzbergen, while, as already mentioned, the diploid race, *E. nigrum*, has a more southern distribution.

Within the *Saxifragaceae* there are several examples of intraspecific chromosome races. *Saxifraga stellaris* was found by various authors to have the somatic chromosome number 28. This number was found by SKOVSTED (1934) in material from Norway, by BÖCHER (1938) in material from Swedish Lapland, and by the present writer in material from Tromsö (arctic Norway). In *S. stellaris* var. *comosa* BÖCHER (l. c.) found $2n = 56$ in material from Lapland, and this number was also found in the present material from Spitzbergen. When the chromosome numbers and the morphology of the chromosomes are taken into consideration, the most obvious conclusion is that var. *comosa* has arisen from *S. stellaris* by chromosome doubling. HARMSEN (1939) also assumes that var. *comosa* is a polyploid form of *S. stellaris* or a closely related species. The number $2n = 64$, which was found by HARMSEN in the embryo of var. *comosa* from Greenland, is no evidence against this assumption (cf. HARMSEN, l. c.). The two forms differ with respect to ecology and geographical distribution. Var. *comosa*, having the higher number of chromosomes, is circumpolar, high-arctic (-alpine), whereas *stellaris* is subarctic — temperate — alpine.

In material of *Saxifraga nivalis* cultivated in the Botanical Garden of the University of Copenhagen SKOVSTED (1934) found the chromosome number $2n = 28$, and in *S. nivalis* var. *tenuis* from Lapland (Sweden) BÖCHER (1938) found the number $2n = 20$. In the examined material from Spitzbergen the numbers found are $2n = 60$ and 20 for *nivalis* and var. *tenuis* respectively. With reference to the number $2n = 28$ found by SKOVSTED, BÖCHER thinks it possible that in this case the basic number is 7 and that the number, $2n = 20$, found in var. *tenuis* may have arisen by a cross between two *nivalis* races having the chromosome numbers $n = 7$ and 14 respectively. The numbers found in the Spitzbergen material, however, furnish stronger support for the assumption that the basic number is 10. — The external morphological characters, which refer essentially to quantitative differences — *nivalis* is more robust —, as well as the fact that no chromosome-morphological differences can be shown between the two forms may indicate that *nivalis* has arisen from var. *tenuis* by autopolyploidy. The polyploid form has a wider area of distribution than the diploid. This is especially true of the vertical distribution in that the polyploid

form goes farther down into the lowland as well as higher up on the mountains than the diploid form. Both forms occur in Spitzbergen, but according to SCHOLANDER (1934) *nivalis* is found somewhat farther north than var. *tenuis*. The two forms are also otherwise different with respect to ecology (cf. ARWIDSSON, 1938).

With regard to *Saxifraga hieraciifolia* from the high-alpine regions of Pamir and Altai, SOKOLOVSKAJA and STRELKOVA (1938) report the chromosome number $2n = 80—82$. On the other hand, the same species from Spitzbergen was found to have $2n = 112$ chromosomes. SOKOLOVSKAJA and STRELKOVA point out that in their investigations they occasionally came across polyploids with higher chromosome numbers in the arctic zone than in the mountainous regions. *S. hieraciifolia*, mentioned above, furnishes a new example of this. In *Saxifraga hirculus* from the same localities, Pamir and Altai, these authors found the chromosome number $2n = 28$, while the chromosome number of this species from Spitzbergen was found to be $2n = 32$.

In material of *Saxifraga oppositifolia* from Norway SKOVSTED (1934) found the number $2n = 26$. The same species from Spitzbergen, on the other hand, was found to have $2n = 52$ chromosomes. Still another example showing that the northernmost representatives have higher chromosome numbers than those having a more southern habitat.

On the question of the correlation between chromosome number and geographical distribution it has been repeatedly pointed out by various authors that it is not an absolute rule that representatives with high chromosome numbers are the most extreme arctic or alpine, and there are also interesting examples of this among the Spitzbergen flora. As already mentioned, we know the extreme instances of this kind as represented by *Vaccinium uliginosum* and *Campanula rotundifolia* from the investigations of material from Greenland and other places carried out by HAGERUP and BÖCHER. Both these species occur as diploids also in Spitzbergen, whereas representatives of the same species growing farther south are tetraploids. A new, analogous case is furnished by *Chrysosplenium alternifolium*. In discussing the typical arctic form, *C. alternifolium* var. *tetrandum*, SCHOLANDER (l. c., p. 18) writes as follows: »It would be of considerable interest to know the chromosome numbers of this little four-staminate form as compared with the southern larger and eight-staminate *C. alternifolium*. It would not be very surprising if the latter may be tetraploid as compared with the former». The cytological examinations now prove that SCHOLANDER's assumption was correct. The chromosome number in var. *tetrandum* from Spitz-

bergen was found to be 2n = 24, while the more southern *alternifolium*, according to SKOVSTED (l. c.), has the number 2n = 48.

Another new example of the same kind is provided by *Cochlearia officinalis*. In material of this species from the S. W. coast of Wales CRANE and GAIRDNER (1923) found the tetraploid number 2n = 28. In *C. officinalis* var. *alpina* from the Faroe Islands BÖCHER (1938) found the divergent number 2n = 26. On the other hand, in material of *C. officinalis* var. *groenlandica* from Spitzbergen, whose habitat is mainly confined to the northernmost polar regions, the diploid number 2n = 14 was found.

These last-mentioned findings need not, however, alter the main impression. The percentage of polyploids in this high-arctic flora is unusually high and, as already pointed out, should be interpreted as an evident tendency indicating that an increased chromosome number increases the adaptability to extreme environmental conditions. *Polyploidy must therefore have played an important role in the origin of the forms or species that have colonized the arctic regions.*

On the subject of polyploid intraspecific chromosome races MÜNTZING (1936) has rectified a lot of important data which affords a foundation for the conclusion that the majority of these races are autopolyploids, and that autopolyploidy or a purely quantitative increase in chromosome number has played a very important role in the evolution of higher plants. The above-mentioned intraspecific chromosome races should supply still further examples to MÜNTZING's already representative list of cases which strictly justifies this conclusion.

In modern floristic literature several of the chromosome races recorded here as varieties of the same species are separated as distinct species. The differences found in chromosome number furnish a new diagnostic character for this differentiation.

SUMMARY.

1. An investigation has been made of the chromosome numbers in higher plants from Spitzbergen. Altogether 68 species and varieties have been investigated. A list of these species and their chromosome numbers is given on pp. 432—434.

2. Of the species and varieties examined 14 are diploids and 54 polyploids, i. e. about 80 per cent polyploids. The high percentage of polyploids among this high-arctic flora agrees very well with the findings of other authors in their investigations of the chromosome

numbers of plants from other regions with extremely unfavourable climatic conditions. This may be interpreted as an evident tendency in the direction that an increased chromosome number increases the adaptability to extreme habitats, like arctic and alpine conditions. Polyploidy should therefore have played an important role in the origin of those forms or species which have colonized such regions as the arctic or alpine.

3. Several instances of intraspecific chromosome races, some of them new, were met with in the Spitzbergen flora. In most cases the high chromosomal race has the most northern range of distribution, but there are also examples showing the reverse of this. New instances of the latter are furnished by *Chrysosplenium alternifolium* and *Cochlearia officinalis*, both of which occur with diploid forms in Spitzbergen, while the more southern forms are polyploids. As has been pointed out by several other authors, this implies that there is no absolute rule that high chromosome number is associated with fitness to extreme habitats.

This investigation was carried out with financial support from »Conrad Holmboe's Research Fund», Tromsö, to which I wish to express my sincere gratitude.

LITERATURE CITED.

1. ARWIDSSON, TH. 1938. Einige neue Gesichtspunkte zu den Chromosomenzahlenbestimmungen. — Svensk Bot. Tidskr. 32, pp. 191—208.
2. BÖCHER, T. W. 1936. Cytological studies on *Campanula rotundifolia*. — Hereditas XXII, pp. 269—277.
3. — 1938. Zur Zytologie einiger arktischen und borealen Blütenpflanzen. — Svensk Bot. Tidskr. 32, pp. 346—361.
4. CRANE, M. B. and GAIRDNER, A. E. 1923. Species-crosses in *Cochlearia*, with a preliminary account of their cytology. — Journ. of Genetics 13, pp. 187—200.
5. FLOVIK, K. 1936. The somatic chromosomes of certain arctic species of the genus *Ranunculus*. — Soc. Sci. Fenn., Comm. Biol. V. 7, pp. 1—18.
6. — 1938. Cytological studies of arctic grasses. — Hereditas XXIV, pp. 265—376.
7. HAGERUP, O. 1927. *Empetrum hermaphroditum* (LGE) HAGERUP. A new tetraploid, bisexual species. — Dansk Bot. Arkiv 5, Nr. 2, pp. 1—17.
8. — 1928. Morphological and cytological studies of *Bicornes*. — Dansk Bot. Arkiv 6, Nr. 1, pp. 1—26.
9. — 1931. Über Polyploidie in Beziehung zu Klima, Ökologie und Phylogenie. — Hereditas XVI, pp. 19—40.
10. — 1933. Studies on polyploid ecotypes in *Vaccinium uliginosum* L. — Hereditas XVIII, pp. 122—128.

11. HARMSEN, L. 1939. Studies on the embryology and cytology of *Saxifraga*. I. — Meddelelser om Grönland 125, Nr. 4, pp. 1—15 (Köbenhavn).
12. MANTON, I. 1932. Introduction to the general cytology of the *Cruciferae*. — Annals of Bot. XLVI, pp. 509—556.
13. — 1934. The problem of *Biscutella laevigata* L. — Zeitschr. f. ind. Abstamm.- und Vererb.-Lehre, LXVII, pp. 41—57.
14. MÜNTZING, A. 1936. The evolutionary significance of autopolyploidy. — Hereditas XXI, pp. 263—376.
15. ROHWEDER, H. 1936. (Cited from TISCHLER, G. 1938. Pflanzliche Chromosomenzahlen IV. — Tab. Biol. XVI, p. 170.)
16. SCHOLANDER, P. F. 1934. Vascular plants from northern Svalbard with remarks on the vegetation in North-East Land. — Skrifter om Svalbard og Ishavet 62, pp. 1—153 (Oslo).
17. SKOVSTED, A. 1934. Cytological studies in the tribe *Saxifrageae*. — Dansk Bot. Arkiv 8, Nr. 5, pp. 1—52.
18. SOKOLOVSKAJA, A. P. and STRELKOVA, O. S. 1938. Polyploidy in the high mountain regions of Pamir and Altai. — Comptes Rendus (Doklady) de l'Acad. des Sci. de l'URSS 21, pp. 68—71.
19. STOCKWELL, P. 1935. Chromosome numbers of some of the *Cactaceae*. — The Bot. Gazette XCVI, pp. 565—570.
20. TISCHLER, G. 1934. Die Bedeutung der Polyploidie für die Verbreitung der Angiospermen. — Bot. Jahrb. 67, pp. 1—36.
21. TURESSON, G. 1938. Chromosome stability in Linnean species. — Annals of the Agric. Coll. of Sweden 5, pp. 405—416.
22. TÄCKHOLM, G. 1922. Zytologische Studien über die Gattung *Rosa*. — Acta Horti Bergiani 7, pp. 97—381.

EINE SERIE MULTIPLER ALLELE FÜR BLÜTENZEICHNUNGEN BEI GODETIA AMOENA

VON *GUNNAR HIORTH*

GENETISCHES INSTITUT DER LANDWIRTSCHAFTLICHEN HOCHSCHULE, AAS, NORWEGEN

I.

GODETIA ist eine beliebte einjährige Zierpflanze, die in zahlreichen Sorten kultiviert wird. Im Samenkatalog der Firma Benary, Erfurt, für das Jahr 1939 werden z. B. 42 Sorten verzeichnet. Abgesehen von der Sorte »Blauer Zwerg», die einer chilenischen Art entstammt, gehören alle diese Typen zu den beiden kalifornischen Arten *Godetia Whitneyi* und *Godetia amoena*. Die Gartenrassen dieser beiden Arten sind im Durchschnitt auffällig voneinander unterschieden:

Godetia Whitneyi.	*Godetia amoena.*
Niedrig — mittelhoch; buschig, stark verzweigt mit dichtem Blütenstand.	Hoch; lockerer, weniger verzweigter Habitus mit lockerem Blütenstand.
Kronblätter nie mit Fleck direkt an der Basis.	In der Regel mit Fleck direkt an der Basis.
Antheren nicht so satt gefärbt.	Antheren satt rot mit sattgelber Spitze.
Kapseln dick, fleischig, erst spät und nur bei günstigem Wetter trocknend, oft völlig geschlossen bleibend.	Kapseln dünn, leicht trocknend, bei Reife weit aufspringend.
	Samen kleiner.

Die Artzugehörigkeit der Gartensorten lässt sich in der Regel leicht bestimmen. Im Samenkatalog von Benary sind z. B. sämtliche 60 cm hohen oder höheren Sorten der Art *amoena*, sämtliche niedrigeren der Art *Whitneyi* zuzurechnen. Zweifel über die Artzugehörigkeit einer Sorte lassen sich übrigens leicht durch Kreuzung entscheiden, da der Bastard zwischen beiden Arten nahezu steril ist.

Die genannten Unterschiede sind jedoch nur bei der Einteilung der Gartenformen verwendbar. Über die Charakterisierung der natürlichen Formen von *Whitneyi* und *amoena* hat bis in letzter Zeit grosse Unsicherheit geherrscht. Kreuzungsversuche mit natürlichen Formen, die ich im Jahre 1936 eingesammelt habe, gestatten indessen, diese Arten genauer zu definieren. Über diese Untersuchungen wird eine besondere Abhandlung erfolgen. Einige Konklusionen seien indessen schon an dieser Stelle mitgeteilt.

Nach der neuesten systematischen Bearbeitung der Gattung *Godetia* von HITCHCOCK (1930) kommt *Godetia amoena* an der Küste des Stillen Ozeans von Monterey (150 km südlich von San Francisco) bis zur Südspitze der Vancouver-Insel in British Columbia, Kanada, vor, während für *G. Whitneyi* nur ein einziger Fundort, Shelter Cove (Humboldt Co), an der Küste des nördlichen Kaliforniens angegeben wird. Dem gegenüber ergaben meine Versuche folgendes:

1) Es besteht eine scharf umgrenzte Gruppe von *Godetia*-Arten, die *amoena-Gruppe*, die mit keiner anderen Gruppe verwechselt werden kann. Es erscheint zweckmässig, diese Gruppe in 3 Arten aufzuteilen, *G. nutans*, *G. Whitneyi* und *G. amoena*. Erstgenannte Art ist durch hängende Blütenknospen charakterisiert, während die beiden letzteren aufrechte Knospen haben. Die Bastarde zwischen Formen mit aufrechten und mit hängenden Knospen sind stets nahezu steril.

2) Die Formen der *amoena*-Gruppe mit aufrechten Knospen sind durch eine geographische Sterilitätsgrenze in zwei Arten geteilt, indem die Formen südlich der Meeresbucht Golden Gate bei San Francisco mit den Formen nördlich von Golden Gate sterile Bastarde geben. Wegen der grossen Variabilität der nördlich von Golden Gate befindlichen Art fällt es dagegen sehr schwer, diese Sterilitätsgrenze mit einwandfreien morphologischen Unterschieden zu korrelieren. Indessen wurde ein eigentümlicher Unterschied in der Blütenzeichnung gefunden. Ein kleiner Fleck *direkt* an der Basis des Kronblattes (*Basalfleck*) ist die vorherrschende Zeichnung südlich von Golden Gate. Ein mehr oder weniger weit von der Basis entfernter Fleck (*Zentralfleck*) herrscht nördlich von Golden Gate vor. Unter zahlreichen Populationen südlich von Golden Gate habe ich nie eine Pflanze mit Zentralfleck angetroffen, unter noch weit mehr Populationen nördlich von Golden Gate nie einen Basalfleck. Individuen ohne jeglichen Fleck kommen dagegen in stark wechselnder Häufigkeit im ganzen Verbreitungsgebiet der *amoena*-Gruppe vor. — Auch in der Antherenfarbe scheint ein gewisser Unterschied zwischen beiden Arten zu bestehen, indem die Rassen südlich von Golden Gate ein leuchtendes Rot mit satt gelber Spitze haben, die Rassen nördlich davon weniger satte Farben.

3) Die kultivierten *Whitneyi*-Rassen geben fertile Bastarde mit den Formen nördlich von Golden Gate, die kultivierten *amoena*-Rassen dagegen mit denen südlich von Golden Gate. Es scheint mir daher zweckmässig, die nördlich von Golden Gate vorkommende Art als *G. Whitneyi*, die südlich davon vorkommende als *G. amoena* zu bezeichnen.

4) Die auffälligen Unterschiede zwischen kultivierten *Godetia*

amoena- und *G. Whitneyi*-Typen dürften zum grössten Teile auf Eigentümlichkeiten der Lokalrasse von *G. Whitneyi* beruhen, die zum Ausgangsmaterial für die Zierpflanzen dieser Art wurde, zum Teil auch auf Auslese bei der Züchtung. Die Zierformen von *amoena* haben in ihrem Habitus relativ grosse Ähnlichkeit mit den natürlichen Rassen dieser Art, während die kultivierten *Whitneyi*-Formen von den meisten wilden *Whitneyi*-Rassen stark abweichen.

Die Versuche mit *Godetia* wurden mit Handelssorten von *Whitneyi* und *amoena* im Jahre 1931 begonnen und 1936 durch Einsammlung wilder Rassen erweitert. Die Versuche mit *amoena* wurden in der Hauptsache auf die Analyse einer Serie multipler Allele für Blütenzeichnungen beschränkt und werden mit der vorliegenden Mitteilung im wesentlichen abgeschlossen.

Als Versuchsobjekte sind *G. Whitneyi* und *G. amoena* etwa gleich bequem. *G. Whitneyi* hat indessen den Vorteil einer weit grösseren Verbreitung in der Natur und einer vielfach grösseren Variabilität. Die Versuche werden nunmehr mit dieser Art allein fortgesetzt. — Es ist indessen wahrscheinlich, dass für genetische Versuche die extrem variable Gruppe *Godetia quadrivulnera-purpurea* noch besser geeignet wäre.

II.

Während ein Zentralfleck in natürlichen *amoena*-Populationen nie angetroffen wurde, kommt er bei einigen Handelssorten dieser Art vor. Dies liesse sich durch folgende Annahmen erklären: Der Zentralfleck könnte in anderen Teilen des Verbreitungsgebietes dieser Art vorkommen. Er könnte durch Kreuzung von den Arten *G. nutans* oder *G. Whitneyi* erworben sein oder innerhalb der Gartensorten durch Mutation entstanden sein. — Bei *G. nutans* wurden sowohl Rassen mit Zentralfleck als auch solche mit Basalfleck angetroffen, allerdings an weit voneinander entfernten Standorten.

Im Folgenden wird gezeigt, dass die Fleckformen der Gartenrassen von *amoena* durch eine Serie von multiplen Allelen bedingt werden. Es wurde mit folgenden Typen gearbeitet:

f Ag = ohne Fleck, Antheren rot.
F^{bsw} *Ag* = schwacher Basalfleck, Antheren rot.
F^{bst} *Ag* = starker Basalfleck, Antheren rot.
F^{k} *ag* = kleiner Zentralfleck, Antheren gelb.
F^{m} *ag* = mittlerer Zentralfleck, Antheren gelb.

F^m *Ag* = mittlerer Zentralfleck, Antheren rot.
F^g *ag* = grosser Zentralfleck, Antheren gelb.

Ag-Pflanzen haben leuchtend rote Antheren mit grösserer oder kleinerer gelber Spitze, *ag* gelbe Antheren oder sehr schwach rote mit

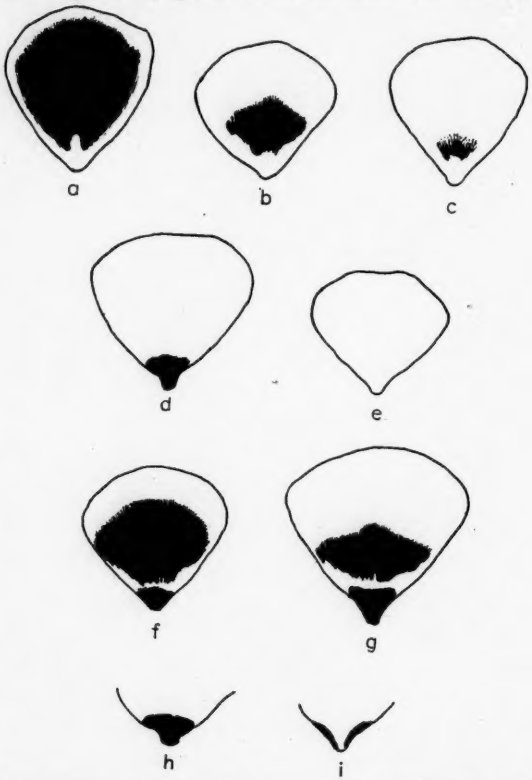


Fig. 1. Kronblattzeichnungen bei *Godetia amoena*. — *a*: Grosser Zentralfleck F^gF^g; *b*: mittlerer Zentralfleck F^mF^m; *c*: kleiner Zentralfleck F^kF^k; *d*: starker Basalfleck $F^{bst}F^{bst}$; *e*: ohne Fleck *ff*; *f*: F^gF^{bst}; *g*: F^mF^{bst}; *h* und *i*: Aussenseite (Unterseite) der Kronblattbasis von F^{bst} bzw. F^{bsw}.

gelber Spitze. Wenn auch die Färbung der »gelben Antheren» etwas variiert, ist der Unterschied zwischen *Ag* und *ag* auffällig und scharf.

f, das nicht aus Gartenmaterial sondern aus einer wilden Rasse stammt, bedingt vollständigen Fleckmangel.

F^{bsw}-Individuen besitzen einen relativ kleinen Fleck an der Basis des Kronblattes, F^{bst} einen etwas grösseren, stärker gefärbten (Fig. 1 *d*).

Die verschiedenen Typen von Zentralfleck sind bei *amoena* etwas näher an der Basis gelegen als bei *Whitneyi*. F^k-Individuen haben einen in Form und Grösse stark variablen Fleck. Dieser besteht aus kurzen roten Streifen, Strichen oder Punkten, die, wenn in geringer Anzahl vorhanden, nur ein kleines gestricheltes Areal bilden, wenn sie zahlreicher und dichter sind, zu einem fast kompakten Fleck zusammenfliessen, der nahezu die Grösse eines mittleren Zentralfleckes erreichen kann. Der durchschnittliche Unterschied zwischen F^k und F^m ist jedoch beträchtlich. Bei den übrigen Typen des Zentralfleckes sind nur die Ränder in Spitzen, Striche oder Punkte aufgelöst, die in der Richtung der Nervatur des Kronblattes verlaufen, welche von der Basis des Kronblattes fächerförmig zum distalen Rand ausstrahlt. Bei F^m-Pflanzen ist der Fleck ziemlich gross und kräftig gefärbt (Fig. 1 *b*). Bei F^g ist er noch grösser, sodass nur schmalere oder breitere Säume an den Rändern des Kronblattes freibleiben (Fig. 1 *a*).

Ausser obigen Allelen dürfte es noch eine Reihe anderer geben, die z. B. intermediäre Fleckgrössen zwischen den oben genannten bedingen.

Genauere Bestimmungen der Farben sind nicht vorgenommen worden. Die Grundfarbe (Farbe des ungefleckten Teils des Kronblattes) ist ein sehr helles Violett, während der Zentralfleck satt rotviolett gefärbt ist. Der Basalfleck hat in der Regel einen etwas abweichenden roten Farbton.

III.

Die Allelie der genannten Gene wurde durch die Kreuzungen in Tabelle 1 bewiesen. Zur Vereinfachung der Darstellung wurde im allgemeinen nicht zwischen F^m *Ag* und F^m *ag*, ferner zwischen F^{bst} und F^{bsw} unterschieden. Die beiden letzteren Allele wurden dann unter der Bezeichnung F^b zusammengefasst. Es wurde jedoch mit allen diesen Typen gearbeitet und ein deutlicher Unterschied zwischen ihnen festgestellt.

Nr. 1—4 (Tabelle 1) zeigen die Spaltungszahlen in F_2 der Kreuzungen $F^g \times f$, $F^m \times f$, $F^k \times f$ und $F^b \times f$. Wir erhalten stets rund ein Viertel *ff*-Individuen. *f* ist jedoch nicht völlig rezessiv, da es in Heterozygoten die Grösse des Fleckes in der Regel etwas verkleinert und den Farbton etwas abschwächt.

F^g dominiert über F^m und lässt sich in F_2 sicher von ihm trennen (Nr. 5). Nr. 10 zeigt das Ergebnis der Rückkreuzung $F^gF^m \times ff$. Die beiden Typen in der Nachkommenschaft, F^gf und F^mf, waren in ihrer

Fleckgrösse so stark verschieden und variierten so wenig, dass jedes Individuum ohne Mühe identifiziert werden konnte. Dieser Befund wurde durch die gleichzeitige Spaltung eines gekoppelten Gens bestätigt; siehe unter Nr. 21.

Nr. 6 enthält eine Kreuzung $F^{bst} \times F^{bsw}$. Starker Basalfleck dominiert über schwachen und in F_2 liess sich jede Pflanze bequem auszählen, was besonders dadurch ermöglicht wird, dass diese beiden Allele eine verschiedene Zeichnung auf der Aussenseite der Kronblätter bedingen (Fig. 1 *h*, *i*).

Kreuzungen von F^g, F^m und F^k mit F^b ergeben F_1-Pflanzen, die

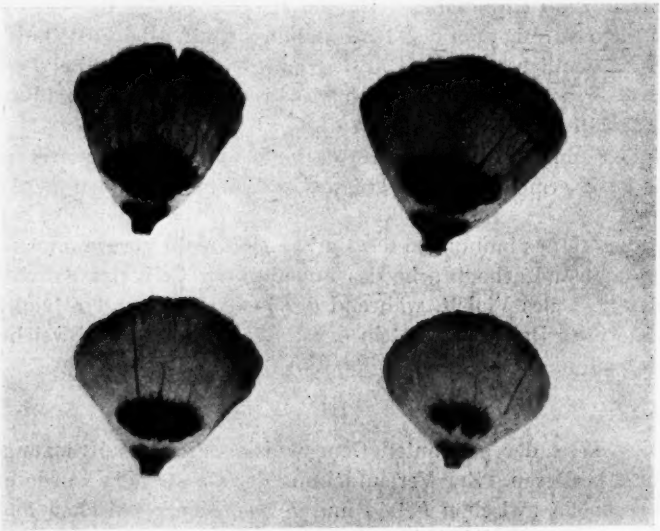


Fig. 2. Kronblätter von F^mF^b-Pflanzen aus F_2 von $F^m \times F^b$. Lichtbild von getrockneten Kronblättern.

beiderlei Flecktypen nebeneinander zeigen. Wurde hierzu F^{bsw} benutzt, so war der Basalfleck meist abgeschwächt und konnte in einigen Blüten fast verschwunden sein. Wurde dagegen F^{bst} verwendet, so zeigten sowohl F^gF^b-, F^mF^b- wie F^kF^b-Pflanzen einen sehr deutlichen Basalfleck (Fig. 1 *f*, *g*). In F_2 (Nr. 7—9) ergab sich eine Spaltung nach dem Schema

$$1\,F^x : 2\,F^xF^b : 1\,F^b$$

wobei F^x den betreffenden Typus von Zentralfleck bezeichnet (Fig. 3).

Eine Nr. 8 entsprechende Kreuzung ist schon von RASMUSON (1921) mit demselben Ergebnis untersucht worden. Da ihm aber innerhalb

der Art *amoena* keine anderen Flecktypen zur Verfügung standen, speziell keine flecklose Rasse, konnte er den Nachweis der Allelie von F^m und F^b nicht vollständig erbringen.

Unter Nr. 11—13 finden wir die Rückkreuzungen der Typen F^gF^b, F^mF^b und F^kF^b mit *ff*. Etwa die Hälfte der Nachkommen hat in jedem Fall den betreffenden Typus des Zentralfleckes, die andere Hälfte einen

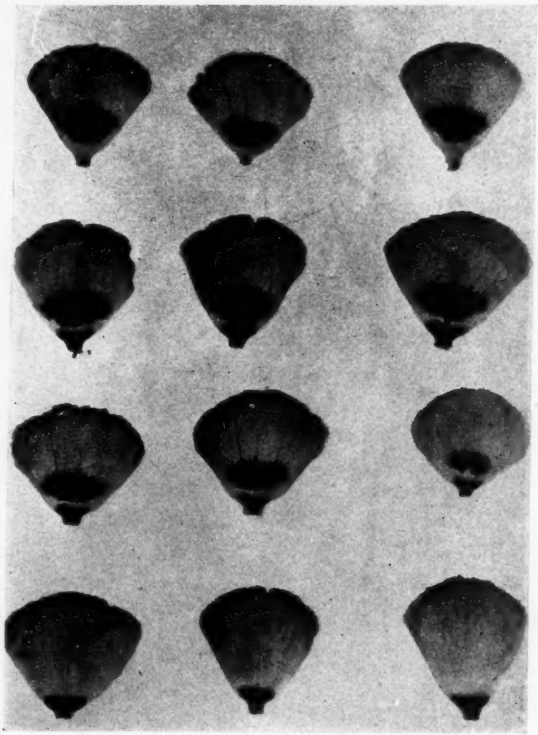


Fig. 3. Kronblätter der Typen F^mF^m, F^mF^b und F^bF^b aus F_2 von $F^m \times F^b$.

Basalfleck. — Wollte man annehmen, dass Basalfleck und Zentralfleck nicht auf allelen sondern auf gekoppelten Genen beruhten, so müsste hier eine besonders starke Koppelung vorliegen. Denn bei den Rückkreuzungen Nr. 11—13 würde ein crossing-over sich durch das Auftreten von F^xF^b- und *ff*-Pflanzen verraten. Unter 3070 Nachkommen wurden keine derartigen Pflanzen gefunden.

Vereinzelte Ausnahme-Individuen mit Zentralfleck + Basalfleck,

TABELLE 1. *Übersicht über die Spaltungen der Blütenzeichnungen bei Godetia amoena. Beschreibung der Merkmale siehe Abschnitt II, S. 443—445* *.

	Kreuzung	F^x	f			n
Nr. 1.	$(F^g f)^2$	159	55			214
» 2.	$(F^m f)^2$	375	89			464
» 3.	$(F^k f)^2$	123	40			163
» 4.	$(F^b f)^2$	608	168			776
		F^g	F^m			
» 5.	$(F^g F^m)^2$	137	32			169
		F^{bst}	F^{bsw}			
» 6.	$(F^{bst} F^{bsw})^2$	106	31			137
		F^x	$F^x F^b$	F^b		
» 7.	$(F^g F^b)^2$	97	207	102		406
» 8.	$(F^m F^b)^2$	223	513	208		944
» 9.	$(F^k F^b)^2$	75	221	93		389
	Σ 7—9	395	941	403		1739
		F^g	F^m			
Nr. 10.	$F^g F^m \times ff$	186	190			376
		F^x	F^b			
» 11.	$F^g F^b \times ff$	605	551			1156
» 12.	$F^m F^b \times ff$	568	532			1100
» 13.	$F^k F^b \times ff$	435	379			814
	Σ 11—13	1608	1462			3070
		$F^x ag$	$F^x F^b Ag$	$F^b Ag$		
Nr. 14.	$(F^g ag/F^b Ag)^2$	35	80	26		141
» 15.	$(F^m ag/F^b Ag)^2$	56	124	49		229
	Σ 14—15	91	204	75		370
		$F^g ag$	$F^g Ag$	fAg		
Nr. 16.	$(F^g ag/fAg)^2$	44	115	55		214
		$F^m ag$	$F^m F^b Ag$			
» 17.	$F^m ag/F^b Ag \times F^m ag F^m ag$	178	216			394
		$F^x Ro$	$F^x ro$	$F^y Ro$	$F^y ro$	
Nr. 18.	$(F^x Ro/F^y ro)^2$ **	334	53	46	82	515
» 19.	$(F^x ro/F^y Ro)^2$	393	190	139	5	727
» 20.	$F^g ro/F^b Ro \times ff\ ro\ ro$	76	230	231	68	605
» 21.	$F^g ro/F^m Ro \times ff\ ro\ ro$	42	144	146	44	376
	Σ 20—21	118	374	377	112	981

die in zwei anderen Rückkreuzungsfamilien von diesem Typus auftraten, besassen, wie eine Nachprüfung zeigte, die Konstitution F^x/F^b und konnten demnach nicht durch crossing-over entstanden sein, sondern dürften auf Versuchsfehler beruhen.

Eine starke Koppelung der Antherenfarbe mit bestimmten Fleck-Allelen wurde in zahlreichen Kreuzungen konstatiert. Nr. 14—17 gibt das relativ geringe Material wieder, in dem die Antherenfarbe genau ausgezählt wurde. In Nr. 17 würde jeder Fall von crossing-over sich durch Entstehung von F^mAg- oder F^mF^bag-Pflanzen verraten, während in Nr. 14—15 nur die Hälfte der Crossovers entdeckt werden könnten. Zusammen befand sich daher unter ca. 580 Gameten kein Crossover-Gamet.

Da *Ag* ungefähr dieselbe rote Farbe auf den Antheren bedingt, wie F^g oder F^m auf den Kronblättern, dürfte es immerhin wahrscheinlicher sein, dass Antheren- und Kronblattfarbe von ein und demselben Gen bedingt werden als von zwei gekoppelten. Es liessen sich demnach auf Grund der Antherenfarbe einige weitere Allele für den Lokus *F* aufstellen; z. B. wären F^mag und F^mAg zwei *F*-Allele. — Übrigens bestärken die Befunde über die Antherenfarbe zugleich auch die Annahme von Allelie zwischen Basalfleck und Zentralfleck. Denn je mehr Gene mit Wirkung auf dasselbe Merkmal (lokalisierte rote Farbe) an derselben Stelle der Koppelungsgruppe gefunden werden, um so grösser ist die Wahrscheinlichkeit dafür, dass die gemeinsame Lage der Gene durch Allelie bedingt wird.

In zahlreichen Kreuzungen wurde schliesslich eine partielle Koppelung konstatiert zwischen den *F*-Allelen und einem Gen *ro* (rosa), das die hell violette Grundfarbe der Blüte in weisslich-rosa verwandelt. In Nr. 18 und 19 bezeichnen F^x und F^y verschiedene Fleck-Allele. Das Rekombinationsprozent (in Nr. 20—21) beträgt $230 : 981 = 23\ \%$.

Was die Konstanz der benutzten Fleck-Allele betrifft, so machen F^g, F^m, F^b und *f* durchaus den Eindruck von stabilen Genen. In den F_2-Familien von Nr. 1—9 und den Rückkreuzungen Nr. 10—13 traten keine Individuen auf, die den normalen Variationsbereich ihres Allels

* F^x bezeichnet den in der Spalte Kreuzung an erster Stelle, F^y den an zweiter Stelle genannten Zeichnungstypus. F^b bezeichnet in einigen Fällen F^{bst}, in anderen F^{bsw}.

** Nr. 18 und 19 fassen eine Reihe von Kreuzungen zusammen, in denen verschiedene, hier nicht angegebene Allele spalten. Zur Vereinfachung der Darstellung wird hier F^y als rezessiv behandelt.

überschritten und etwa eine für ein anderes Allel typische Fleckgrösse zeigten. Indessen haben F^k und ein bestimmter, nicht näher daraufhin untersuchter Typus von F^m eine recht variable Fleckgrösse. Für F^k wurden nicht unbeträchtliche Unterschiede festgestellt zwischen verschiedenen Blüten einer Pflanze, verschiedenen Pflanzen einer Familie und zwischen verschiedenen Familien. Der Fleck kann winzig klein sein oder nahezu die Grösse eines mittleren Zentralfleckes erreichen. Der durchschnittliche Unterschied zwischen F^m und F^k ist jedoch sowohl bei Selbstungen als auch in F_2 bei Kreuzungen mit den gleichen Linien recht auffällig. Es liegt nahe anzunehmen, dass F^k ein labiles Gen ist, das innerhalb bestimmter Grenzen variiert.

Eine detaillierte Besprechung der Zahlenverhältnisse hat wenig Interesse, da bei *Godetia* statistisch bedeutsame Abweichungen nicht selten sind. Es dürfte genügen, auf einen Befund hinzuweisen. In Σ 7—9 treten die Heterozygoten in einer Häufigkeit von 54,1 % statt $50 \pm 1{,}2$ % auf. Abweichung: mittlerer Fehler $= 3{,}4$. Auch in Nr. 14—16 scheint die Häufigkeit der Heterozygoten erhöht zu sein. Da eine Begünstigung bestimmter Gametentypen stets zu weniger als 50 % Heterozygoten führen würde, muss hier nach einer anderen Erklärung gesucht werden. Man könnte annehmen, dass die Homozygoten mit zwei *F*-Chromosomen gleichen Ursprunges eine etwas stärkere Inzuchtswirkung zeigen und daher eine höhere Sterblichkeit haben als die Heterozygoten mit zwei *F*-Chromosomen verschiedenen Ursprunges.

IV. DISKUSSION.

Zwischen den Gliedern der Serie F^g, F^m, F^k, F^{bst}, F^{bsw}, f bestehen teils quantitative, teils qualitative Unterschiede. F^g, F^m und F^k bedingen verschiedene Grössen des Zentralfleckes; ein Zentralfleck ist aber durch seine Lage qualitativ von einem Basalfleck unterschieden.

Bei Kreuzungen quantitativ verschiedener Zeichnungen erhalten wir die üblichen Dominanzverhältnisse, der grössere Fleck ist (nahezu) dominant über den kleineren. Bei Verbindung qualitativ verschiedener Zeichnungen dagegen gibt es keine Dominanz, sondern die Heterozygoten zeigen beiderlei Flecktypen deutlich nebeneinander. Im Prinzip dürfte die Sachlage die gleiche sein, wenn die Kombination $F^m ag\ f\ Ag$ neben dem Zentralfleck von F^m die roten Antheren von f zeigt. In diesen Fällen wirken also die betreffenden Allele unabhängig voneinander, genau so wie es von mir (1931) bei qualitativ verschiedenen Blatt- und Kotyledonenzeichnungen bei *Collinsia* gefunden wurde.

Man könnte theoretisch erwarten, beim Studium von in der Natur vorkommenden Allelenserien relativ oft auf eine »unabhängige Wirkung» von Allelen zu stossen, indem die verschiedenen Allele einer bestimmten Serie durch eine Anzahl von Mutationsschritten, die im Laufe der Evolution des Gens nach und nach im selben Lokus stattgefunden haben, sich in stark divergierende Richtung entwickelt haben könnten (vgl. HIORTH, 1931, S. 262—266). Dies scheint auch der Fall zu sein. Verf. ist in drei Fällen auf eine unabhängige Wirkung von Allelen gestossen, nämlich bei Blatt- und Kotyledonenzeichnungen von *Collinsia*, bei den hier besprochenen Blütenzeichnungen von *Godetia amoena* und bei noch näher zu untersuchenden Kotyledonenzeichnungen von *Godetia Whitneyi*.

Inwiefern ein Zentralfleck innerhalb der Art *G. amoena* als natürlicher Charakter aufgefasst werden darf, bleibt allerdings nach den Angaben auf S. 443 fraglich. Daher sind in diesem Zusammenhang die Verhältnisse bei der verwandten Art *G. nutans* von Interesse, bei der sowohl Standorte mit Basalfleck, mit Zentralfleck und ohne Fleck vorkommen. Fleck gekreuzt mit ohne Fleck gibt 3 : 1-Spaltungen. Die Kreuzung einer Form mit Basalfleck (Magalia, Butte Co, Calif.) mit einer mit Zentralfleck (Glen Ellen, Sonoma Co, Calif.) ergab in F_2 eine Spaltung: 16 mit Zentralfleck : 46 Zentralfleck + Basalfleck : 21 mit Basalfleck, also ein Verhältnis 1 : 2 : 1. Demnach sind anscheinend auch bei dieser Art die Gene für Basalfleck und Zentralfleck allel und zeigen unabhängige Wirkung.

Dagegen sind nur wenig entsprechende Fälle in der Literatur erwähnt. Dies dürfte aber wohl darauf beruhen, dass für die meisten anderen Charaktere als lokalisierte Zeichnungen eine unabhängige Wirkung von Allelen nicht ohne besondere Untersuchungen nachgewiesen werden kann. Die »intermediäre Vererbung», die so allgemein bei Kreuzungen natürlicher Rassen angetroffen wird, liesse sich vielleicht bei genauester Betrachtung zum grossen Teil auf eine unabhängige Wirkung von Allelen zurückführen.

EAST (1936, S. 392) fand bei seinen Untersuchungen über Artbastarde bei *Nicotiana* weder Anzeichen dominanter noch rezessiver Gene, sondern es schien, dass »each gene is an active pattern former».

EAST unterscheidet zwischen defectiven und non-defectiven Allelen und führt die Heterosiserscheinungen hauptsächlich auf letztere zurück. Während in der Regel zwei *identische* non-defective Allele *AA* bei Homozygoten die gleiche Wirkung hätten wie ein einziges bei Heterozygoten *Aa*, könnten zwei *verschiedene* non-defective Allele (z. B. A_1A_2 oder A_3A_4) eine kumulative Wirkung entfalten. »The cumulative action of two non-defective allelomorphs of a given gene approaches the strictly additive as they diverge from each other in function.»

Es wäre interessant das *Godetia amoena*-Material im Sinne dieser Theorie auszuwerten. Dieses Versuchsobjekt scheint beträchtliche Vorteile für derartige Studien zu haben, insbesondere den, dass in den meisten benutzten Linien keine modifizierenden Gene vorkamen, die die Wirkung der Fleck-Allele in stärkerem Grade beeinflussen. Es war jedoch keine Gelegenheit, das Material eingehend genug zu studieren. Ein interessanter Befund liess sich indessen auch ohne Spezialuntersuchungen feststellen. Die Heterozygoten F^kF^{bst} haben durchschnittlich einen weit stärkeren Zentralfleck als F^kF^k-Pflanzen. Hier liegt anscheinend nicht nur eine additive Wirkung zweier Allele vor, sondern auch eine darüber hinaus gehende Verstärkung.

V. ZUSAMMENFASSUNG.

1) Der Artname *Godetia amoena* wird hier auf die Rassen südlich von Golden Gate (San Francisco) mit aufrechten Knospen beschränkt.

2) Bei Gartenrassen von *Godetia amoena* wurde eine Serie multipler Allele für Blütenzeichnungen festgestellt. F^g, F^m, F^k bedingen einen grossen bzw. mittleren, kleinen »Zentralfleck», F^{bst}, F^{bsw} einen starken bzw. schwachen »Basalfleck», *f* vollständigen Mangel des Fleckes. Siehe Fig. 1 *a—e*.

3) Die fünf ersteren Allele sind unvollständig dominant über *f*; F^g ist (nahezu) dominant über F^m.

4) Die Kombinationen von F^g, F^m und F^k mit F^{bst} und F^{bsw} zeigen beiderlei Zeichnungstypen nebeneinander. Siehe Fig. 1 *f*, *g*; Fig. 2. *Bei Kreuzungen qualitativ verschiedener Zeichnungen wird also keine Dominanz, sondern eine »unabhängige Wirkung» der Allele angetroffen.* — F^{bst} vergrössert den Zentralfleck von F^k.

5) Rote (statt gelbe) Antherenfarbe ist stark mit bestimmten Fleck-Allelen gekoppelt. Unter ca. 580 Gameten wurde kein Fall von crossing-over angetroffen. Falls, wie es wahrscheinlich ist, rote Antherenfarbe und rote Blütenzeichnungen durch dasselbe Gen bedingt werden, muss die Anzahl der *F*-Allele erhöht werden.

6) Die *F*-Allele sind mit einem Gen für rosa-weisse Grundfarbe der Blüten gekoppelt mit einem Rekombinationsprozent von 23.

Die vorliegenden Untersuchungen sind durch Beiträge von norwegischen wissenschaftlichen Fonds (A/S Norsk Varekrigsforsikrings

Fond, Norges Landbrukshöiskoles Forskningsfond) ermöglicht worden. Hierfür sei auch an dieser Stelle mein bester Dank ausgesprochen.

Aas, Norwegen, 20. Januar 1940.

ZITIERTE LITERATUR.

1. East, E. M. 1936. Heterosis. — Genetics, 21, 375—397.
2. Hiorth, G. 1931. Genetische Versuche mit *Collinsia*. II. Die Blatt- und Kotyledonenzeichnungen von *Collinsia bicolor*. — Zeitschr. f. indukt. Abst.- u. Vererbungsl. 59, 236—269.
3. Hitchcock, C. L. 1930. Revision of North American species of *Godetia*. — Bot. Gaz., 89, 321—360.
4. Rasmuson, H. 1921. Beiträge zu einer genetischen Analyse zweier *Godetia*-Arten und ihrer Bastarde. — Hereditas, II, 143—289.

MEIOSIS OF ALLIUM PORRUM, A TETRAPLOID SPECIES WITH CHIASMA LOCALISATION

BY *ALBERT LEVAN*
CYTO-GENETIC LABORATORY, SVALÖF, SWEDEN

WHILE meiosis of diploid forms with localised chiasmata has been analysed on several occasions within various Liliaceous genera (*Fritillaria*, *Allium*, *Trillium*), this characteristic course of meiosis has not been studied so far in any polyploid form. Such a study affords an approach to problems of very great interest. DARLINGTON (1937) points to one special problem: »In polyploid forms with localised pairing and chiasmata we must expect fewer multivalents than in corresponding forms with complete pairing, since the effective length for pairing is shorter» (l. c. p. 129). This conclusion is drawn from a study of diploid *Fritillaria* species, where the pachytene pairing in forms with localisation of chiasmata only reaches about 50 % of the total length of the chromosomes. In *Allium*, on the other hand, the pachytene pairing is morphologically complete in localised species such as *Allium fistulosum* (LEVAN, 1933), it is complete even in asynaptic species, where no single chiasma is formed. Thus, in an autopolyploid *Allium* with localised chiasmata a normal frequency of multivalents may be expected at earlier meiotic stages, and this frequency should decrease during the course of the prophase. At metaphase I only such multivalents as are associated by chiasmata should be left, even if the mutual orientation of some bivalents should indicate that earlier they had been joined to quadrivalents.

During my work on the cytology of *Allium* I came across an autotetraploid species, *Allium Porrum* L., which showed almost complete localisation of chiasmata at meiosis. I studied several forms of the species, both Swedish commercial varieties and material procured from botanical gardens. All these forms were tetraploids and had localised chiasmata. In one form I found a regular occurrence of two small somatic chromosome fragments, which sometimes paired at meiosis, in other respects no cytological differences were found between the different forms.

In *Allium Porrum* ordinary NAVASHIN fixations of whole buds, preceded by a short dipping in CARNOY, gave better results than smears in osmic acid fixations. The pachytene stage in particular is very beautiful in NAVASHIN fixed material, permitting a detailed study of whole quadrivalents at this stage.

The somatic chromosomes of *Allium Porrum* are of the ordinary *Allium* type, 28 chromosomes have a median centric constriction and 4 chromosomes have a subterminal constriction. These latter are the s_1 chromosomes and their satellite is quite small. The length of the longest chromosomes of *Allium Porrum* is about 10 μ.

I. THE COURSE OF MEIOSIS.

As already mentioned, the prophase stages show complete chromosome pairing. Small unpaired segments may, of course, be found even at mid-pachytene, but they do not occur more frequently towards the chromosome ends. Such unpaired segments are found also in species with random-distribution of chiasmata. The chromomeric structure is very clear at pachytene, and the chromomeres are sometimes rather broad, almost band-like. There is one nucleolus in each cell. At the nucleolus two deeply stained lumps, evidently the end portions of the s_1 bivalents, may be seen quite regularly. No differences in the pairing can be seen between the proximal and the distal parts of the chromosomes. The diplotene loops, however, seem to appear at first in the distal parts of the chromosomes, and this might be an indication of a less intimate pairing in these parts.

During pachytene quadrivalents are easily found in most cells (Fig. 1 *a—b*). The exchange of threads within the quadrivalents usually occurs in one place, but also quadrivalents with two exchanges have been met with. If there is one exchange it may be located in any region of the chromosomes, medially or terminally. It is difficult at this stage to analyse whole cells, but I have often observed more than one quadrivalent in the same cell. Sometimes all the chromosomes seem to be joined into 8 quadrivalents.

At diplotene the quadrivalents are still present in great number (Fig. 1 *c—d*). The spiralisation is prominent and the quadrivalents are held together by the torsion. During diakinesis, however, many of the earlier quadrivalents are seen to fall apart into pairs of bivalents (Fig. 1 *e—h*). Now the remarkably regular distribution of chiasmata is clearly seen: always 2 chiasmata per bivalent, one at each side of

the centromere. Owing to the size of the chromosomes and their great number diakinesis is not a suitable stage for studying the quadrivalents.

At the first metaphase the chromosomes are arranged regularly into an equatorial plate. The characteristic structure of the bivalents is now clear. Owing to the chromosome contraction, the two chiasmata of each bivalent have been pulled together close to the centromere, so the chromosomes seem to touch each other only at one point. In

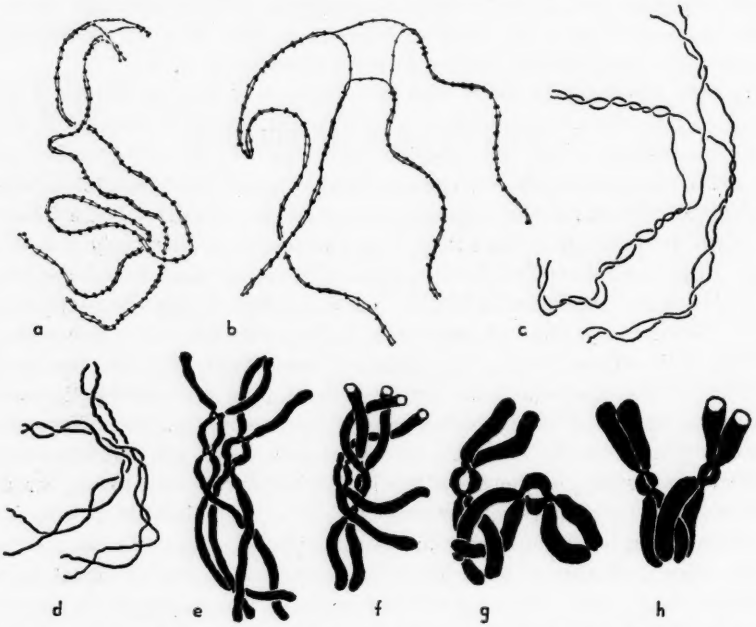


Fig. 1. *a—b*: quadrivalents at pachytene, *c*, *d*: at diplotene, *e—h*: quadrivalents at diakinesis, held together only by torsion. — × 3900.

polar view (Fig. 2 *a*) it is difficult to see the chiasmata but in side-view (Fig. 2 *b*) the chromatid arrangement is made quite clear. Most plates consist of only this kind of bivalents. The regularity is striking and gives a good illustration of DARLINGTON's term »semi-clonal» inheritance.

There may occur, however, certain exceptions to the scheme. The most common exception is the occurrence of bivalents with only one chiasma (Fig. 2 *c—d*). In one pollen sac the following frequency of such bivalents was counted:

Number of deviating bivalents:	0	1	2	3	4	5	Total	M/cell
Number of cases:	7	6	9	3	5	1	31	1,9

Compared with the conditions in *Allium fistulosum*, a diploid species with chiasma localisation, the frequency of deviating bivalents is rather high. *Allium fistulosum* had from 0,15 to 0,55 such bivalents per cell. In the next chapter it is suggested that the formation of pachytene

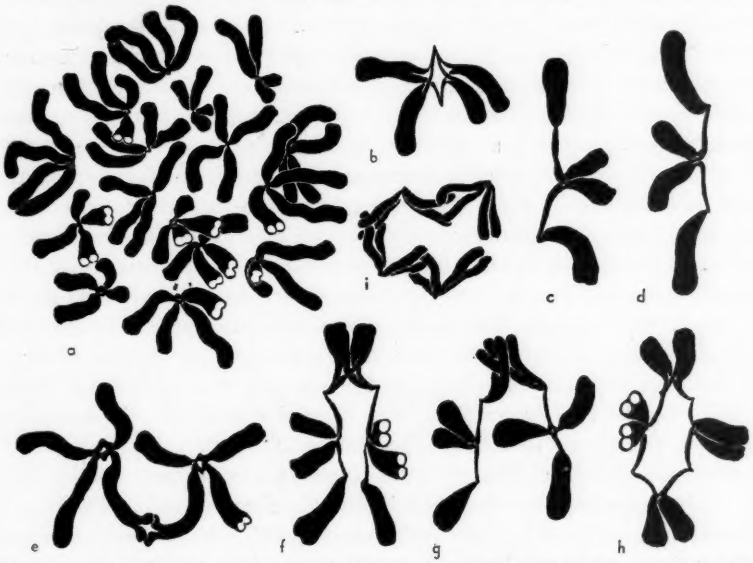


Fig. 2. *a*: metaphase I, a plate consisting of 16 cruciform bivalents seen from the pole, *b*: side-view of a cruciform bivalent, *c*, *d*: bivalents with one chiasma, *e*: a quadrivalent held together by a non-localised chiasma, *f*, *g*: localised chain quadrivalents, *h*: a localised ring quadrivalent, *i*: a ring quadrivalent at anaphase I. — *a*: × 1400, *b*—*i*: × 2800.

quadrivalents may predispose to the origin of pairs of bivalents with one chiasma.

In these bivalents with only one chiasma, usually the portion between the centromeres and the chiasma is extended and is somewhat narrower than the rest of the chromosomes. The chiasma is often more terminalised than in the cruciform bivalents within the same plate. The chromatid pairing evidently yields more easily under the strain from the centromeres in the bivalents with only one chiasma. In the cruci-

form bivalents, where four chromatids resist the strain of the centromeres, the chiasmata seem to be more stationary.

Quadrivalents are present so rarely at metaphase I that an intense study was needed merely to demonstrate their occurrence. This study was made difficult by the large size of the 64 long cross-arms present, which quite fill up the equatorial plates, so that the exact analysis of each element is often rendered impossible. It was detected, however, that now and then two cross-arms joined by a clear-cut chiasma were stretched out extremely far towards one pole. In the cases where the connections of these arms could be followed also inside the plate they were found to form each one chiasma with two other chromosomes. In fact there were present instances of chain quadrivalents of a type hitherto unrecorded (Fig. 2 *f—g*). The chiasmata of these quadrivalents were gathered as close to the four centromeres as possible. After an extensive search the corresponding ring-type of quadrivalents was also found (Fig. 2 *h*). These rings were observed only three times. I wish to point out, however, that the occurrence of quadrivalents is probably somewhat more frequent than these data indicate, since they can be demonstrated only in especially favourable cases. Even if this is taken into account the quadrivalents must be very rare at metaphase I, and certainly much rarer than in autotetraploids with random-distribution of chiasmata. I counted in one slide 6 quadrivalents in 250 analysed cells and in another slide 4 quadrivalents in 130 cells. In normal tetraploids of *Allium* the average frequency is 1—5 quadrivalents per cell, i. e. about 100 times greater frequency.

The appearance of the quadrivalents is characteristic. They differ from ordinary quadrivalents in the same qualities as localised bivalents differ from ordinary bivalents. Thus the chiasmata of the quadrivalents are very close to the centromeres, which brings about the formation of large cross-arms at each chiasma. The orientation on the spindle of the quadrivalents is typical, the 4 centromeres form a rectangle with 2 corners at each side of the equator. Neighbouring centromeres are orientated towards the same pole. Anaphase formations indicate that sometimes also zig-zag arrangement may occur, but such quadrivalents were not observed at metaphase. Anaphase of a ring quadrivalent is seen in Fig. 2 *i*.

It was often noticed that in the quadrivalents the portions between the centromeres and the chiasmata were longer and more extended than the corresponding portion of the cruciform bivalents. The chiasmata of the quadrivalents were evidently more terminalised. This is

probably due to the same condition as that causing the greater terminalisation of the bivalents with one chiasma. It may be that also quadrivalents with their centromeres more close together really occurred, they would anyhow be very difficult to analyse. Formations which might be such quadrivalents were sometimes noticed, but they were interpreted as interlockings of two bivalents.

In the same manner as in *Allium fistulosum*, the localisation of the chiasmata was not absolute, non-localised chiasmata were observed also in *Allium Porrum*, although very seldom. Such chiasmata could give rise to quadrivalents of a new type. They were built up of two bivalents in which two cross-arms, one from each bivalent, were joined to a subterminal chiasma (Fig. 2 *e*). This chiasma did not influence the orientation of the quadrivalent on the spindle. The frequency of non-localised chiasmata was lower than in *Allium fistulosum*, where some forms had up to 1,7 non-localised chiasmata per cell. In one slide of *Allium Porrum* 3 such chiasmata were counted in 250 cells, and their average occurrence probably does not exceed 1 % of the cells.

Anaphase I and the second division take place normally. The pollen grains contain 16 chromosomes, so the few quadrivalents of meiosis evidently give rise to but slight disturbances.

II. DISCUSSION.

In the present paper a record is given of meiosis of an autotetraploid *Allium* species with almost absolute localisation of the chiasmata. DARLINGTON (1939) regards this proximal type of localisation only as a special case. Its type depends on the location of the original segment of pairing at zygotene. In other cases, for instance, tetraploid species of *Tradescantia*, chiasmata are formed only at the chromosome ends, due to the fact that the pairing starts distally.

Another independent genetic variable determining the meiosis type is the time limit of effective pairing. The time limit determines the degree of localisation. If the time of effective pairing is unlimited, the chromosomes will be paired along their whole length and their chiasmata will be distributed at random. The reason why the ends of the chromosomes of the proximal type of localisation do not form any chiasmata is, still according to DARLINGTON, not due to a precocious reproduction of the chromosomes in these parts, as HUSKINS and SMITH (1934) suggested, but is instead due to the time limit, which cuts off the effective pairing before it has reached the whole chromosome length.

The shorter this time limit, the more absolute is the localisation of chiasmata. In such asynaptic forms as *Allium amplectens* (LEVAN, 1940) the time limit must be nil. Since, however, a clear morphological pachytene pairing is seen both in localised and asynaptic *Allium* species,

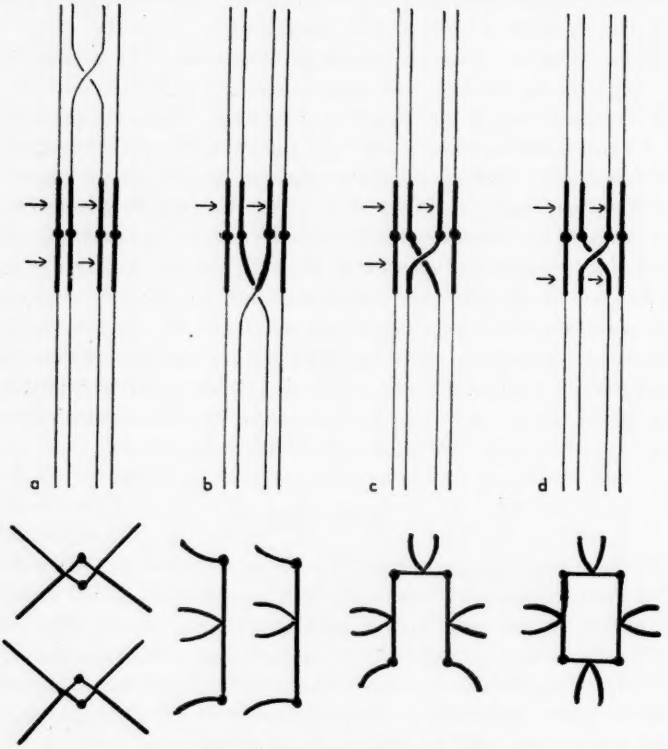


Fig. 3. Scheme of the possible cases of partner exchange in the pachytene quadrivalents and the result at metaphase I. Chiasmata are formed at the arrows. For further explanation see the text.

a distinction must be made between the apparent, visible pairing and the effective pairing, i. e. the pairing which gives rise to chiasmata.

It is clear that the original segment of pairing in such a species as *Allium Porrum* cannot be localised to a single exact point of the four homologous chromosomes. In that case quadrivalents could hardly be formed. The frequent occurrence of quadrivalents at pachytene indicates, in my opinion, that the pairing starts in different places even in

chromosomes with absolute localisation of chiasmata. A quadrivalent can be formed only if the pairing starts in at least two places within different pairs of the 4 homologous chromosomes. These starting points are possibly located close to the centromeres in *Allium Porrum*, otherwise the conclusion must be drawn that the zone of effective pairing is not always the zone of the earliest pairing.

However this may be, it is certain that quadrivalents are formed in a greater number at pachytene than survive until metaphase. In later stages chiasmata are found only on both sides of the centromeres. If we suppose, in accordance with DARLINGTON, that chiasmata arise at the same place as they are found at metaphase I the following possibilities are valid within each four-group of homologous chromosomes (see Fig. 3, where each pachytene chromosome is represented by a thickly drawn central portion corresponding to the zone of effective pairing, and thinner end-portions, where chiasmata are formed only in exceptional cases). If the exchange of partners can occur anywhere within different parts of the chromosomes, it is evident that the commonest case will be Fig. 3 *a*, where the exchange occurs outside the central portion. This leads to the formation of 2 typical, localised bivalents, which in earlier stages are often joined by a torsion pairing, but which are free at metaphase I. If the exchange of partners takes place close enough to the central zone (Fig. 3 *b*), it is probable that the exchange will interfere with the pairing of the central zone on that side of the centromere, so that chiasmata can be formed only on the other side of the centromere. The result will be 2 bivalents with one chiasma each. This is probably the cause of the relatively high frequency of this kind of bivalents in *Allium Porrum*, as compared with diploid species with localised chiasmata. If in the former case the pairing is inhibited only in one pair of threads there will originate a localised chain quadrivalent with 3 chiasmata (Fig. 3 *c*). Finally, if the exchange of partners occurs exactly at the centromeres, so that chiasmata can be formed on both sides of the exchange, a localised ring quadrivalent will result. It is quite comprehensible that this last case must be very rare, since it depends for its realisation on the fulfilment of quite special conditions.

SUMMARY.

Meiosis is examined in *Allium Porrum*, an autotetraploid species with complete localisation of chiasmata. Quadrivalents are formed

rather frequently at zygotene, but most of them disappear before metaphase I is reached. Chiasmata are formed only in the neighbourhood of the centromeres. A new type of metaphase quadrivalents is described: chains and rings with localised chiasmata.

Svalöf, January 20th, 1940.

LITERATURE CITED.

1. Darlington, C. D. 1937. Recent advances in cytology. 2nd Ed. — London.
2. — 1939. The evolution of genetic systems. — Cambridge.
3. Huskins, C. L. and Smith, S. G. 1934. Chromosome division and pairing in *Fritillaria Meleagris*: The mechanism of meiosis. — Journ. of Gen. 28: 397—406.
4. Levan, A. 1933. Cytological studies in *Allium*, IV. *Allium fistulosum*. — Svensk Bot. Tidskr. 27: 211—232.
5. — 1940. The cytology of *Allium amplectens* and the occurrence in nature of its asynapsis. — Hereditas XXVI: 353—394.

THE MODE OF CHROMOSOME PAIRING IN PHLEUM TWINS WITH 63 CHROMOSOMES AND ITS CYTOGENETIC CONSEQUENCES

BY *A. MÜNTZING* AND *R. PRAKKEN*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

I. INTRODUCTION.

As described in previous publications (MÜNTZING, 1937 a, 1938 a), twin seedlings with deviating chromosome numbers were obtained in 13 species belonging to 11 different genera. This material also included *Phleum pratense* L., which was represented by six triploid and three haploid aberrants. Instead of the normal number, $2n = 42$, these plants had $2n = 63$ and 21 respectively. For the sake of simplicity the aberrants will be referred to in the following as triploids and haploids instead of using the more adequate terms enneaploid and triploid, the normal plants of the species in reality being hexaploid ($2n = 42$).

The present paper is restricted to the results of a study of aberrants with $2n = 63$ and their progeny. Some preliminary data concerning this work have already been given (MÜNTZING, 1937 a, 1938 b and ÅKERMAN, GRANHALL, NILSSON-LEISSNER, MÜNTZING and TEDIN, 1938), but the results now accumulated justify a fuller account.

The experimental work and the somatic chromosome counts were performed at Svalöf in the years 1935—1939 under the guidance of the senior author. The meiotic studies were undertaken at Lund after the arrival of the junior author [1]. Last year the triploid twins and their progenies were handed over to Dr. A. LEVAN, now head of the Cytogenetic Department of the Svalöf Plant Breeding Institute. The haploids will be studied and described by Miss H. NORDENSKIÖLD.

II. THE PROPERTIES OF THE TRIPLOID TWINS.

1. MORPHOLOGY AND FERTILITY.

Habitually the triploid twin plants were rather similar to their diploid sister twins, but a more detailed study revealed certain typical

[1] At present Fellow of the Rockefeller Foundation.

differences. In one of the three twin pairs studied the triploid member was distinctly more vigorous than the diploid sister plant (Fig. 1), in the second pair both members had about the same vigour, and in the third pair the triploid plant seemed to be inferior in vigour in comparison with the diploid sister individual. Small clones were made of

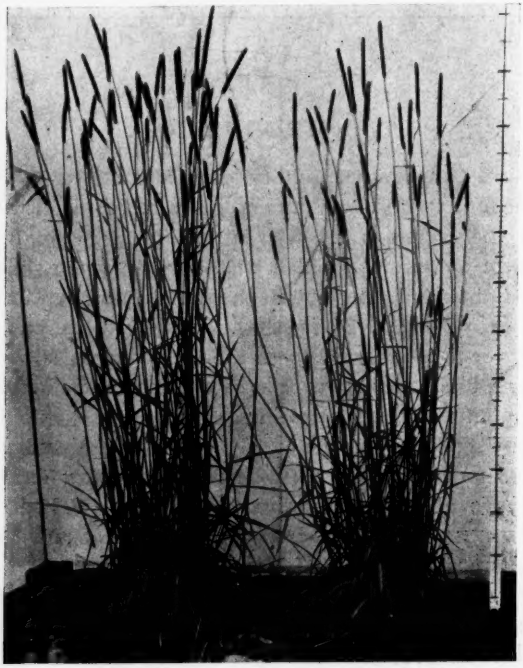


Fig. 1. A twin pair of *Phleum pratense*. The plant to the left is triploid ($2n = 63$) and slightly more vigorous than the diploid sister twin to the right ($2n = 42$).

the six individuals, and in 1938 the average plant weight in these clones (the sum of two cuttings) was found to be the following.

Field number	Chromosome number	Number of clone plants	Average plant weight
4198 *b*	42	8	224 gr.
a	63	7	240 »
2041—2 *a*	42	4	88 »
b	63	4	103 »
4039 *a*	42	4	148 »
b	63	4	131 »

The differences between the twin pairs are evidently much greater than the differences within each pair. On an average, however, the triploid twins seem to be equal or even superior to the diploid normal plants, but more data are needed in order to prove that definitely.

TABLE 1. *Morphological data from twin clones of Phleum pratense* [1].

Field number	Chromosome number	Leaf breadth	Leaf length	Thickness of 10 leaves	Stem diameter	Culm breadth	Culm length	Spikelet length
4198 *b* ...	42	6,19 (16)	31,6 (16)	2,24 (8)	1,10 (24)	8,08 (24)	9,71 (24)	4,13 (24)
» *a* ...	63	6,93 (14)	33,0 (14)	2,66 (7)	1,31 (21)	9,17 (21)	10,10 (21)	4,62 (21)
2041—2 *a*	42	6,25 (8)	29,0 (8)	2,03 (4)	0,94 (12)	6,40 (12)	8,00 (12)	4,00 (12)
» —2 *b*	63	7,63 (8)	29,3 (8)	2,68 (4)	1,16 (12)	7,72 (12)	10,00 (12)	4,00 (12)
4039 *a* ...	42	6,63 (8)	26,0 (8)	2,23 (4)	1,18 (12)	7,72 (12)	10,00 (12)	4,50 (12)
» *b* ...	63	8,25 (8)	31,4 (8)	2 68 (4)	1,30 (12)	8,71 (12)	12,08 (12)	5,75 (12)

The diploid and triploid twins were further compared by a series of measurements, the results of which are given in Table 1. According

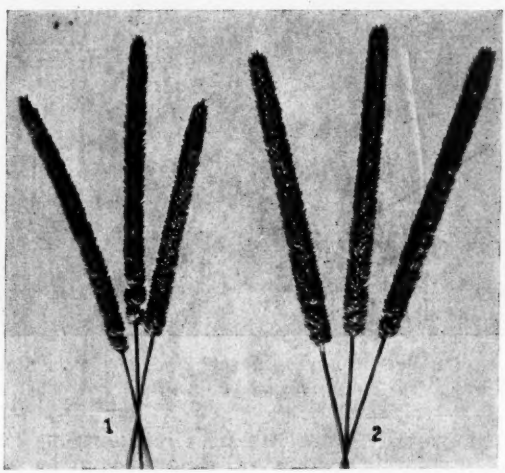


Fig. 2. Culms of the twin pair represented in Fig. 1. The culms of the triploid (to the right) are somewhat bigger than those of the diploid (to the left).

to these data the triploids have longer, broader and thicker leaves than the corresponding diploids and are also characterized by thicker stems,

[1] The number of measurements for each average value between brackets. For leaf and culm length the units are cm., for the other characters mm.

longer and thicker culms and bigger spikelets. The typical differences in culm and spikelet dimensions may also be seen from Figs. 2—3.

Though only three diploid-triploid twin pairs were available for comparison, the differences observed are most probably typical of material of this kind. Rather similar results were also obtained from measurements of twin clones in *Poa pratensis* (MÜNTZING, 1940). The larger dimensions of the triploid *Phleum* plants are indeed to be ex-

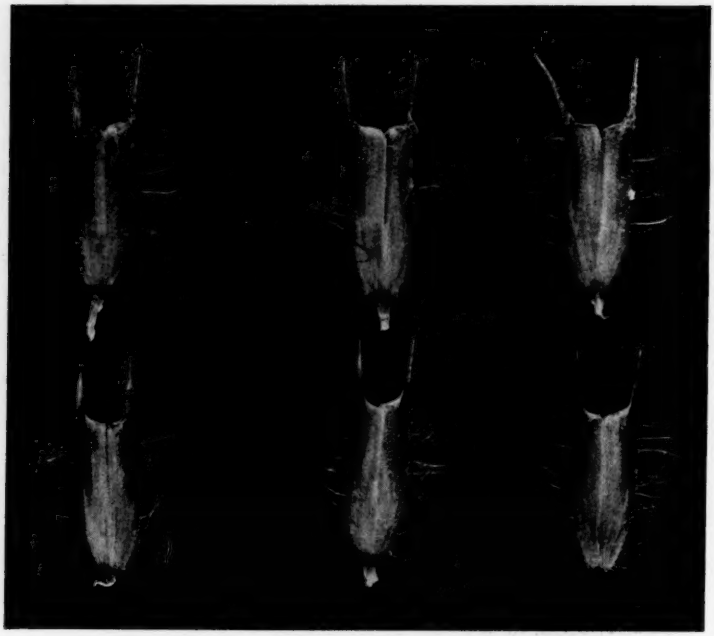


Fig. 3. Spikelets of triploid *P. pratense* (upper row) and of the diploid sister twin (lower row). The dimensions of the former are somewhat larger.

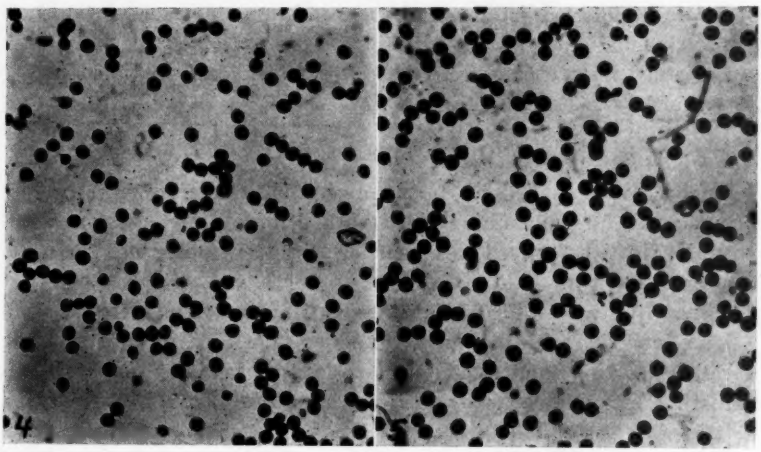
pected by analogy with similar changes in numerous cases of auto-polyploidy previously studied (cf. MÜNTZING, 1936, 1937 b).

Much to our surprise pollen fertility in the triploid members of the twin pairs proved to be just as good as in the corresponding diploids, the percentage of good pollen being almost 100 per cent in all the plants examined. The appearance of pollen samples from one of the twin pairs is evident from Figs. 4—5. The only difference to be seen is a difference in size, the pollen grains of the triploid being slightly larger

than those of the corresponding diploid. The same size differences were also observed in the other two twin pairs. Measurements were undertaken in one of the pairs, the following result being obtained:

	Pollen diameter									
	10	11	12	13	14	15	16	n	M ± m	σ^2
Diploid (4039 *a*) ..	1	5	25	92	34	4		161	$13{,}03 \pm 0{,}06$	0,65
Triploid (4039 *b*) ..				26	39	70	13	148	$14{,}47 \pm 0{,}07$	0,78

The average values are evidently significantly different. The relation between the diameters is $14{,}47 : 13{,}03 = 1{,}111$, which would



Figs. 4—5. Pollen samples from a diploid—triploid twin pair of *P. pratense*. In both twins pollen fertility is quite good, but in the triploid (Fig. 5) the pollen grains are larger than in the diploid (Fig. 4).

correspond to a relation of $1{,}111^3$ or 1,37 between the average volumes. The relation between the chromosome numbers of the mother plants is $63 : 42 = 1{,}50$. Owing to elimination of chromosomes at meiosis (cf. below) the relation between the average chromosome numbers of the two categories of pollen grains will be somewhat lower than 1,50. Thus, in the present material there seems to be a rather close correspondance between chromosome number and pollen grain size.

Pollen size in the triploid seems to be slightly more variable than in the diploid, the variance values being 0,78 and 0,65 respectively. However, the odds that this difference in variability is significant are only

about 5 : 1, as may be controlled by the tables of FISHER and YATES (1938).

A greater variability in size of the pollen grains produced by the triploid was expected, since these pollen grains must contain a variable number of chromosomes. It seemed remarkable that the possible difference in variability was not much greater. However, an explanation of the unexpectedly uniform and good pollen of the triploids was obtained from a study of meiosis in this material.

Before passing on to a description of meiosis, it should only be mentioned that fertility on the female side seems to be just as good as on that of the male. No exact data on seed setting in the triploid twins are yet available, but judging from mere observations seed production in the triploids is as good as in the diploids. At any rate, much seed was obtained from crosses between different triploids, and also selfing gave a fairly good result.

2. MEIOSIS.

Meiosis was studied in the p. m. c. The material was fixed in chrome-acetic-formalin and stained with gentian violet. Though not ideal, the fixations proved to be sufficiently good to disclose the main features of meiosis.

Since the twins with 63 chromosomes are autotriploid in relation to the sister twins with the normal number 42, meiosis was expected to be rather irregular and to be characterized by a high number of trivalents and univalents. However, contrary to expectation, meiosis in the triploids was found to be remarkably regular, the number of trivalents and univalents being low and the number of bivalents high. This was evident from a study of diakinesis and first metaphase (Figs. 6—15).

Fig. 6 represents a diakinesis from the diploid twin. The 42 chromosomes appear as 21 bivalents. In most of the bivalents the chromosomes are joined by two or more chiasmata, but in some bivalents there is only one chiasma. There is no evidence that the chiasmata are localized.

In the triploid twin studied (4198 *a*) attempts were made to analyse a number of complete chromosome complements at diakinesis. This could be done more or less accurately in seven cells, but due to the high number of chromosomes and a somewhat unsatisfactory fixation, there were a few obscure points in each cell. However, it was immediately obvious that most of the chromosomes were united to bi-

valents. In addition to these there were a few univalents and some quite rare trivalents. Two such nuclei are represented in Figs. 7—8.

In Fig. 7 the chromosome complement probably consists of $2_{III} + 27_{II} + 3_{I}$. The trivalents are indicated by single-headed arrows.

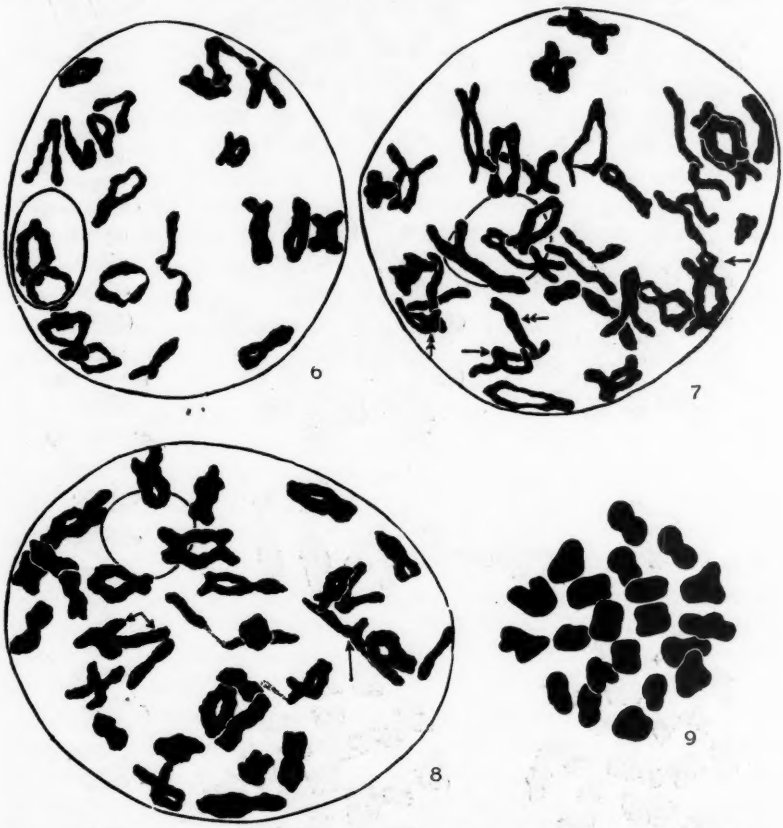
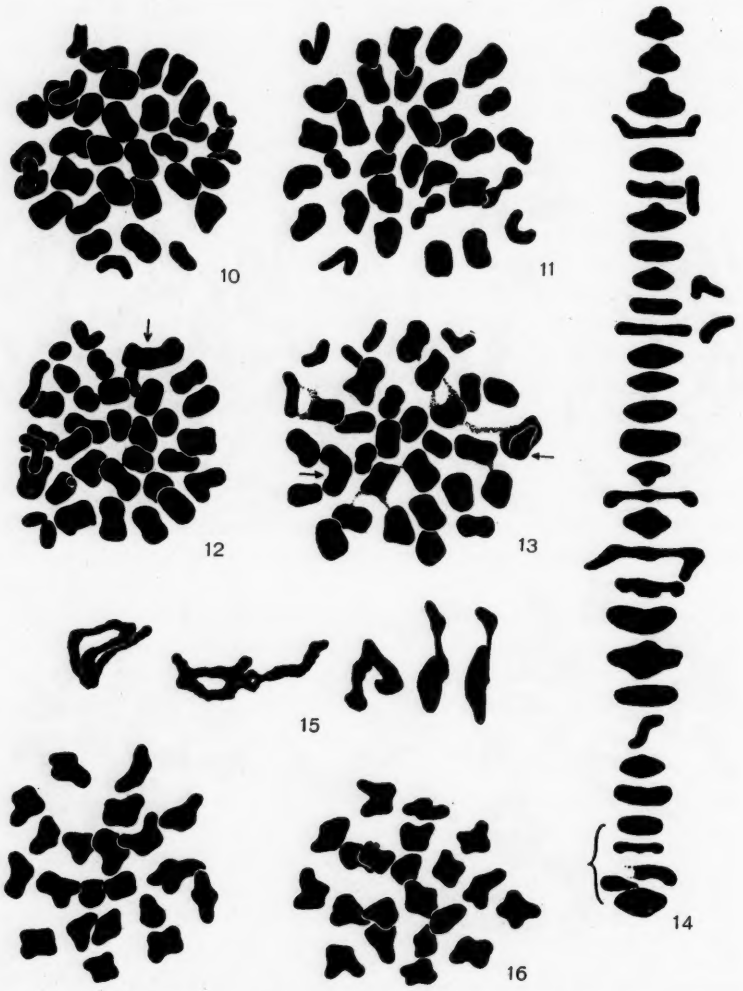


Fig. 6. Diakinesis in diploid *Phleum pratense* ($2n = 42$), 21_{II}. Figs. 7—8. Diakinesis in triploid *Phleum pratense* ($2n = 63$). Fig. 7, probably $2_{III} + 27_{II} + 3_{I}$; Fig. 8, probably $29_{II} + 5_{I}$. Fig. 9, I—M (polar view) of diploid *P. pratense*, 21_{II}. — 4170 ×.

One of them is rather clear, the other one is somewhat more dubious. The clear one is separately drawn in Fig. 15 (the second configuration from the left). Of the 27 bivalents 24 are quite distinct and unquestionable. Of the remaining three, two (indicated by double-headed arrows)



Figs. 10—14. I—M groups in triploid *Phleum pratense*. Fig. 10, $28_{II} + 7_I$; Fig. 11, probably $30_{II} + 3_I$; Fig. 12, probably $1_{III} + 27_{II} + 6_I$; Fig. 13, probably $2_{III} + 26_{II} + 5_I$; Fig. 14, side view (separately drawn), probably $1_{III} + 28_{II} + 4_I$ (the bracket indicates some not quite clear elements). Fig. 15, five separate trivalents from diakinesis (the two to the left) and first metaphase (the three to the right). Fig. 16, I—A in diploid *P. pratense* (polar view), distribution 21—21. — Fig. 14 and the three I—M trivalents in Fig. 15 drawn at a magnification of 3470 ×, the other figures at × 4170.

had not quite clearly visible contours, and in one case (the bivalent just to the right of the nucleolus) the presence of a chiasma was not quite certain. However, judging from the shape and position of the chromosomes, they most probably constitute a bivalent and not two univalents. Of the three univalents one (at 2 o'clock) is quite free, but the other two (at 8 o'clock near the nucleolus) form a cross. The arms of this cross, however, do not seem to be connected.

Since the true configurations at diakinesis are of importance, a second cell is represented in Fig. 8. The probable configuration in this cell is $29_{II} + 5_{I}$. No certain trivalent could be distinguished. 27 bivalents are quite clear but at two points (indicated by arrows) the associations are somewhat obscure. The arrow at 3 o'clock points to a probable chiasma, but it is not excluded that the two chromosomes represent 2 univalents instead of a bivalent. The other (double) arrow indicates a chromosome complex which has been counted as $1_{II} + 2_{I}$, though other interpretations may be possible. The grey connection between the two univalents in the centre is probably an artefact.

In the same way 5 other diakinesis configurations from the same plant were analysed more or less successfully. In these additional cells the following probable configurations could be distinguished: Cell 1, $2_{III} + 27_{II} + 3_{I}$ (possibly only 1_{III}); Cell 2, $27_{II} + 7_{I}$; Cell 3, $24_{II} + 5_{I}$ + unclear group with $\pm 3_{II}$; Cell 4, $26_{II} + 4_{I}$ + an unclear group; Cell 5, $28_{II} + 4_{I}$.

In the last four cells all the 63 chromosomes could not be distinguished, but nevertheless the data are mentioned in order to show that in all cells the number of clear bivalents was higher than 21, the number of univalents never higher than 7 and the frequency of trivalents very low.

The results obtained from the study of diakinesis were verified by observations of *first metaphase* (Figs. 9—15). In the normal twin with 42 chromosomes 21 bivalents were found to be present at I—M (Fig. 9). In the triploid *the sum of the bivalents and the occasional trivalents was hardly ever found to be lower than 28 and the number of univalents was never higher than seven.* This is evident from the following figures.

Fig. 10 shows a I—M in polar view with $28_{II} + 7_{I}$. The univalents are scattered round the bivalents and may be distinguished rather easily. In another I—M group (Fig. 11) the number of bivalents was higher, the number of univalents being correspondingly lower. The probable configuration in this cell is $30_{II} + 3_{I}$. In other cells the

presence of one or two trivalents was rather clear. In Fig. 12 the configuration is probably $1_{III} + 27_{II} + 6_{I}$. However, the trivalent (indicated by an arrow) was not quite certain. One of the univalents has a rather marked median constriction. — In the metaphase group represented by Fig. 13, finally, the probable configuration is $2_{III} + 26_{II} + 5_{I}$, but the trivalents are somewhat uncertain. The greyish streaks connecting some of the chromosomes are probably artefacts.

The chromosome configurations at I—M are also illustrated by Fig. 14, showing the result of an attempt to analyse a complete chromosome complement in side view. 25 bivalents and 4 univalents could be distinguished quite clearly. In addition to these were some additional less clear bivalents and one probable trivalent. Though all the chromosomes could not be seen with accuracy, it is nevertheless evident that the great majority of the chromosomes are present as typical bivalents. A few of them are rod-shaped, but most of them have two or more chiasmata. Though trivalents seem to be quite rare at I—M, a few associations of this kind were really observed (Fig. 15, to the right).

The figures discussed above represent only a small part of the evidence gathered. The total data obtained may be summarized in the following way.

In the diploid twin ($2n = 42$) 3 cells in polar view were found to contain 21 bivalents. In 4 cells in side view 20—21 bivalents could also be distinguished, and there was no evidence of larger associations than bivalents. Of 80 bivalents observed in side view 28 (i. e. $35{,}0 \pm 5{,}3$ per cent) were rod-shaped, the majority being ring-shaped with 2 or more chiasmata [1].

In the triploid ($2n = 63$) attempts were made to analyse 5 different I—M groups in side view, but in none of them could the complete chromosome complement be distinguished. However, the following numbers of bivalents were visible: 27, 25, 23, 28 and 27 respectively. In the same cells the number of univalents observed varied between 4 and 7. A total of 4 more or less clear trivalents were also observed, no cell containing more than one trivalent.

The proportion of rod-shaped bivalents was found to be about the same as in the normal twin. Of 130 bivalents studied 38 belonged to this category, the others being rings with at least 2 chiasmata. This gives

[1] It is possible that the true proportion of ring bivalents is slightly higher. The value mentioned is based on counts in a few, particularly clear I—M groups, in which the proportion of rod bivalents may be somewhat higher than the average. The same is true of the corresponding counts in the triploid.

a percentage value of $29{,}2 \pm 4{,}0$. The difference between this value and the corresponding percentage of the diploid $35{,}0 \pm 5{,}3$ is not significant. At any rate, the triploid is *not* characterized by a higher frequency of rod bivalents than the diploid.

In the triploid a total of 11 cells in polar view were studied. In seven of these the total number of chromosomes was found to be 63. These cells represented the following configurations: $28_{II} + 7_{I}$ (3 cells), $1_{III} + 27_{II} + 6_{I}$ (2 cells); $2_{III} + 26_{II} + 5_{I}$ (1 cell) and $30_{II} + 3_{I}$ (1 cell). In the remaining 4 cells one chromosome was lacking, the total number of chromosomes distinguished being 62. Though these configurations are incomplete the configurations observed may be mentioned, viz. $28_{II} + 6_{I}$ (2 cells); $1_{III} + 27_{II} + 5_{I}$ (1 cell) and $1_{III} + 26_{II} + 7_{I}$ (1 cell). In the last-mentioned cell the sum of the bivalents and trivalents is most probably not higher than 27. This is the only exception so far observed to the rule that the sum of bivalents and trivalents in this material is not lower than 28.

The more or less successful analysis of entire I—M groups was supplemented by counts of the number of univalents per cell. Counts in four different slides gave the following total results:

	Number of univalents						Number of cells	M ± m
	2	3	4	5	6	7		
Polar view.....		2	4	7	10	6	29	$5{,}48 \pm 0{,}22$
Side view	2	9	15	30	24	9	89	$5{,}03 \pm 0{,}12$

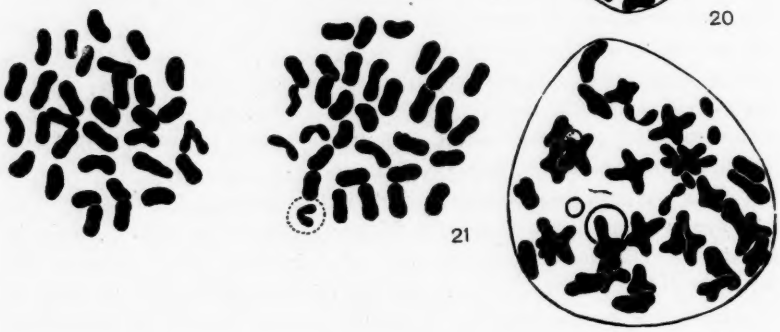
Since some univalents may have been overlooked, the numbers counted represent minimum values. However, the difference between the true and the calculated average values must be relatively slight, the univalents generally lying more or less peripherically and thus being easy to distinguish. At any rate, the true average value is lower than 7, some cells certainly having less than 7 univalents. It is still more important, however, that not a single one of the 118 cells studied was found to contain more than 7 univalents.

When comparing diakinesis and first metaphase the frequency of univalents seems to be somewhat higher at the latter stage. In seven diakinesis cells the probable number of univalents varied from 3 to 7 with an average of 4,43. On inspection of a larger number of cells at diakinesis the impression was obtained that the number of univalents at this stage was in fact slightly lower than at first metaphase.

17

18

19 20


21

First anaphase is quite regular in the diploid, 21 chromosomes going to each pole. In some cells the position of the chromosomes was found to be so regular that the corresponding chromosomes in the two anaphase groups could be distinguished without difficulty (Fig. 16). First anaphase in the triploid (Figs. 17—18) is also rather regular, though minor complications arise due to the presence of univalents. Most of the univalents present at I—M lag and split at I—A, but some of them, lying far from the equator, pass to the poles undivided. The division of the univalents takes place at late anaphase and has not yet occurred in the cells represented by Figs. 17—18. In Fig. 17 the I—M configuration has evidently been $29_{II} + 5_{I}$ (or possibly $2_{III} + 26_{II} + 5_{I}$). At I—A three of the univalents are lagging, but the remaining two (indicated by arrows) are situated below the lower of the anaphase groups and would certainly be included in the interphase nucleus without division. The 29 chromosomes in the two anaphase groups have a corresponding position, which makes it possible to decide rather accurately which chromosomes have been united as bivalents at I—M. The same corresponding position may also be seen rather well in the anaphase chromosomes of Fig. 18. In this cell the two anaphase groups contain 28 chromosomes, 7 chromosomes being situated between the groups. All these 7 chromosomes are probably former I—M univalents, though the possibility is not quite excluded that some of them might be delayed members of previous bi- or trivalents. However, the strict correspondance of the members in the two anaphase groups represents evidence against such an interpretation.

The number of univalents dividing at late I—A was counted in four slides with the following total result:

Number of dividing univalents:	0	1	2	3	4	5	6	7	n	M ± m
» » cells:	2	4	8	8	12	9	4	2	49	$3{,}57 \pm 0{,}25$

The average number of dividing univalents is evidently significantly lower than the number of univalents present at I—M ($5{,}48 \pm 0{,}22$ in

Figs. 17—18, I—A in triploid *Phleum pratense* (polar view). Fig 17, distribution 29—3—31 (two elements in the right group, indicated by arrows, are undivided univalents lying at a deep level). Fig. 18, distribution 28—7—28. — Fig. 19, early interphase in diploid *P. pratense*, 21 chromosomes in each nucleus. Fig. 20, early interphase in triploid *P. pratense*. (In the lower nucleus 29 chromosomes + 5 half univalents, in the upper nucleus 30 chromosomes + 3 half univalents.) Fig. 21, II—M in triploid *P. pratense*. (In the left group probably 31 chromosomes + 2 half univalents; near the right group one eliminated half univalent; in the right group probably 29 chromosomes + 3 half univalents.) — × 4170.

polar view and $5{,}03 \pm 0{,}12$ in side view). This confirms the observation that a proportion of the univalents pass to the poles without division.

The fate of the univalents and the chromosome distribution could be further studied at *interphase*, the chromosomes at the early part of this stage being clearly visible (Figs. 19—20). A typical interphase from the triploid is represented by Fig. 20, which may be compared with the corresponding stage in the diploid (Fig. 19). In Fig. 19 there are, as expected, 21 chromosomes in each cell, in Fig. 20 the situation is more complicated. In the lower nucleus there are 29 ordinary chromosomes and probably 5 half univalents (one of these apparently divided into two parts, due to the pronounced median constriction). In the other nucleus there are 30 ordinary chromosomes and 3 half univalents. This result shows that the two halves of a split univalent may sometimes be included in the same interphase nucleus.

At interphase the proportion of eliminated chromosomes was found to be quite low. Thus, even the products of the lagging and splitting univalents are in most cases included in the nuclei. In 78 cells observed, the number of eliminated chromatids was found to be the following:

Number of eliminated chromatids:	0	1	2	3	n	M
» » p. m. c.:	52	11	13	2	78	0,55

The eliminated chromatids were generally present as half univalents, but in some cases the two chromatids of a univalent had not separated. In most of the pollen mother cells, however, there was no chromosome elimination at this stage, the average number of eliminated chromatids being as low as 0,55.

At interphase the chromosome distribution was studied in ten cells, including the one described above (Fig. 20). In six of these cells it was possible with more or less accuracy to distinguish the split univalents from the other chromosomes. In these cells the distribution was the following: $(29 + 5/2)$ — $(30 + 3/2)$ (Fig. 20); $(29 + 4/2)$ — $(31 + 2/2)$; $(28 + 5/2)$ — $(31 + 3/2)$; $(30 + 2/2)$ — $(31 + 2/2)$; $(29 + 2/2)$ — 2/2 eliminated — $(32 + 0)$; $(29 + 2/2)$ — 2/2 eliminated — $(30 + 2/2)$. In the last-mentioned cell a total of only 62 chromosomes could be distinguished. In 4 additional cells the split univalents could not with certainty be distinguished from the other chromosomes. The number of elements in the two nuclei of these p. m. c. were 32—33, 32—35, 31—32 and 34—35 respectively. Since the total number of elements in these cells varies from 63 to 69, this represents additional evidence that

the univalents splitting at I—A are often included in the interphase nuclei.

At *second metaphase* the split univalents could be distinguished rather well from the other chromosomes. In Fig. 21 the metaphase group to the left most probably contains 31 ordinary chromosomes + 2 half univalents (at 3 o'clock). The other group seems to be composed of 29 chromosomes + 3 half univalents. One half univalent is eliminated. Thus, in this case 3 univalents have evidently divided at first anaphase.

The number of half univalents lying more or less scattered outside the II—M plates was counted in 22 p. m. c. The number of such elements per II—M plate was found to vary between 0 and 5 with an average of 2,32. Since the average number of univalents dividing at I—A was found to be 3,57 the majority of the split univalents evidently lie more or less scattered round the II—M plates.

At *second anaphase* lagging chromosomes were frequent, these laggards undoubtedly being represented by the split univalents. The number of lagging chromosomes was counted in 50 cells, the result being the following:

Number of lagging chromosomes:	1	2	3	4	5	6	n	M
» » cells:	4	11	8	14	8	5	50	3,52

The average number of lagging chromosomes closely corresponds to the average number of univalents dividing at I—A, the two values being 3,52 and 3,57 respectively. Thus, it is highly probable, as is usual in such cases, that univalents dividing at I—A and being included in the daughter nuclei appear as laggards at II—A. A small proportion of the split univalents is eliminated already in the first division, but most of the elimination evidently takes place at II—A.

An attempt was made to estimate the degree of elimination by observations of the number of eliminated chromosomes in young tetrads. These eliminated chromosomes were in a few cases lying in clear micronuclei but generally apparently free in the plasm. In most cases the eliminated bodies evidently correspond to one chromatid (= half a univalent), but sometimes apparently to two chromatids. In a few cases the chromosomal nature of the eliminated bodies was doubtful. Even if, for these reasons, the counts are not quite accurate, they nevertheless represent the true situation fairly well. The following numbers were found:

Number of eliminated chromatids:	0	1	2	3	4	5	n	M
» » tetrad cells:	34	50	28	24	7	1	144	1,47

The average value allows a calculation of the probable average number of chromosomes in the male gametes of the triploid plants. This would be $63/2 - 1{,}47 = 30{,}03$. If the same degree of elimination occurs in the ovules, and if there is no selective zygotic elimination, the average chromosome number of the offspring would be 60,06. At any rate, owing to the observed elimination of chromosomes, the average chromosome number in the offspring should be lower than 63. It might also be predicted that in the progenies of the triploid twins the chromosome number should only in very exceptional cases be lower than 56, practically all gametes receiving at least 28 chromosomes. As described below these predictions were indeed verified.

III. PROGENIES OF TRIPLOID TWINS.

1. MATERIAL.

In 1936 the three twins with 63 chromosomes (Nos. 4198 *a*, 2041—2 *b* and 4039 *b*) flowered for the first time. The culms produced were in part isolated, in part cross-pollinated. Seed after isolation was obtained from 4198 *a* and 4039 *b* and after crossing from the combinations 4198 *a* × 4039 *b* and reciprocally, 4039 *b* × 2041—2 *b* and 2041—2 *b* × × 4198 *a*. The seeds obtained were quite good and were observed to be larger than ordinary timothy seeds. They were germinated in April, 1937 on a germination apparatus, the seedlings were planted in boxes with sterilized soil and later on transplanted to the field. Germination was quite good, the percentage values being 99 for the seed obtained after isolation (122 seedlings from 123 seeds) as well as for the cross-pollinated seed (1589 seedlings from 1611 seeds). As a standard seed of the commercial variety »Gloria» ($2n = 42$) was germinated at the same time. The percentage of germination of the standard was 98. In the field the plants were planted in rows with the distances 40 cm. between the plants and 60 cm. between the rows. Every 10th plant in the rows was a standard plant, thus allowing a rather good comparison between standard and the material to be tested.

2. CHROMOSOMAL VARIATION.

Since the offspring of the triploid plants was represented by as many as 1711 seedlings, it was not possible to determine the somatic chromosome number in all this material. However, a rather good idea of the chromosomal variation was gathered from counts in a total of

186 individuals. The majority of the fixations (148) were made at the seedling stage, care being taken to avoid selection of special seedlings. Later on 38 fixations were also made from especially vigorous plants in the field. The results of the chromosome counts are given in Table 2.

The main result of the chromosome counts is the fact that *of the 186 individuals tested not a single one had a lower chromosome number than 56*. This is a verification of the meiotic observation that practically all gametes receive at least 28 chromosomes. The kind of chromosomal variation in the offspring is also in harmony with the observed mode of meiosis. The numbers found range from 56 to 64, thus most of the

TABLE 2. *Chromosome numbers in the offspring of triploid Phleum pratense* ($2n = 63$).

Field number		Somatic chromosome number 56	57	58	59	60	61	62	63	64	n	M	σ^2
37—2	Without selection	3	1	6	10	8	7	1	3	1	40	59,63	
—3		2	2	5	10	8	9	2	2	4	44	60,02	
—4		1	2	2	5	6	3	2	5	1	27	60,15	
—5		4	3	6	8	11	3	1	1		37	59,00	
Total		10	8	19	33	33	22	6	11	6	148	59,73	3,87
37—1	Selected vigorous plants	3	2	1	—	1					7	57,14	
—2			1	1							2	57,50	
—3		1	—	2	6	2	3	1	4	2	21	60,52	
—5			1	2	—	2	—	1			6	59,17	
—6					1	—	1				2	60,00	
Total		4	4	6	7	5	4	2	4	2	38	59,50	5,45
Total of all values		14	12	25	40	38	26	8	15	8	186	59,66	

plants had numbers lower than those of the mother plants. A calculation of percentage values gives 87,6 per cent with lower numbers than the mother plants, 8,1 per cent with the same number and only 4,3 per cent with a higher number.

All the progenies are similar in this respect, the progenies raised after isolation behaving in the same way as those obtained after crossing. The same range of chromosome variation was also found among the selected vigorous plants as among those taken at random.

The total average chromosome number in the progenies is 59,66, this value being 3,34 lower than 63, the chromosome number of the mother plants. The average decrease is certainly due to the chromosomal elimination occurring at meiosis in the mother plants. According

to the meiotic data given above (p. 478) the average chromosome number of the gametes was calculated to be 30,03. The union of such gametes would result in an average chromosome number of 60,06. The agreement between expectation (60,06) and observation (59,66) is evidently quite good.

For control a few chromosome counts were also undertaken in the standard variety »Gloria». Eight plants were examined and, as expected, found to have $2n = 42$, the normal chromosome number of *Phleum pratense*.

3. PLANT WEIGHT.

The material available allowed a rather accurate comparison between the progenies of twin plants with $2n = 63$ and the commercial variety »Gloria», having the normal chromosome number 42. The first thing to be studied was vigour and productivity as measured by plant weight. The field plants were cut and weighed four times (once in 1937, twice in 1938 and once in 1939). The sum of these weighings may be considered to be a good expression of vigour in this material. The results obtained are summarized in Table 3.

TABLE 3. *Plant weight in the offspring of Phleum pratense twins with 63 chromosomes.*

Field number	Sum of 4 weighings (in hg.) 0	2	4	6	8	10	12	14	16	18	20	n	M ± m (in gr.)	σ^2
37–1	8	32	52	34	25	11	12	1	3			178	658 ± 24	2,89
standard		3	2	4	5	3	2	1				20	830 ± 70	2,98
37–2	2	2	9	24	22	9	6	3	1			78	844 ± 36	2,33
standard		2	1	1	5	4	1					14	858 ± 84	2,34
37–3	10	33	80	155	138	115	36	13	7			587	832 ± 14	2,31
standard		7	7	18	11	10	12	2	—	1		68	876 ± 38	3,12
37–4	2	7	13	6	3							31	506 ± 56	1,10
standard				1	—	2	—	1				4	1100 ± 157	2,67
37–5	12	31	60	113	108	75	39	9	3			450	820 ± 15	2,47
standard		10	6	10	5	13	5	3	1	1		54	860 ± 43	4,20
37–6	2	12	18	12	3							47	508 ± 46	0,95
standard			1	1	2	—	1					5	860 ± 140	2,20
37–1→6, total	36	117	232	344	299	210	93	26	14			1371	787 ± 8	2,56
standard, total		22	17	35	28	32	21	7	1	2		165	867 ± 25	3,30

The first fact to be seen from the table is that the total yield of the

six families is less good than that of the standard, the average values being 787 ± 8 and 867 ± 25 gr. respectively. The difference is significant (D/m = 3,12). However, among the six families there are considerable differences, some of them having about the same average value as the standard, others being quite inferior. The best families are those involving the mother plant 4198 *a*, which had previously been observed to be much more vigorous than the other two twins with 2n = 63 (4039 *b* and 2041—2 b). Progeny 2 is the offspring of isolation of 4198 *a*, and though timothy is a plant species which is rather sensitive to inbreeding (SYLVÉN, 1929; VALLE, 1931), the average value 844 ± 36 is almost as high as that of the corresponding standard, 858 ± 84. The difference is not significant. In three other progenies, 37—1, 37—3, and 37—5, the mother plant 4198 *a* is also involved. Two of these represent crosses 4198 *a* × 4039 *b* (37—3) and reciprocally (37—5), and in these progenies the average values are quite good and not significantly lower than those of the standard. In the third family (37—1) the average vigour is rather poor and most probably lower than in the standard (D/m = 2,32). This family is derived from the cross 2041—2 *b* × × 4198 *a*. Evidently this cross combination is rather unfavourable, the plant 2041—2 *b* being rather poor. It is also possible that a considerable proportion of the plants are the result of self-fertilization, the self-sterility of the mother plants (in this case 2041—2 *b*) not being complete. The two remaining families (37—4 and 37—6) are very poor, and this is not surprising, since they do not involve the vigorous triploid twin 4198 *a*. 37—4 is derived from isolation of 4039 *b*, and 37—6 represents the cross 4039 *b* × 2041—2 *b*.

In spite of the fact that there is a good deal of chromosome variation in the progenies of triploid twins, these progenies appeared just as uniform in the field as the cytologically stable standard variety. In fact, the variance values of the twin progenies were all found to be *lower* than those of the corresponding standard plants. This, however, may be due to the fact that the standard plants were growing at wider distances from each other than the other plants, thus being more exposed to the soil variation. At any rate, the variability in the twin progenies, in spite of the chromosomal diversity, was of about the same order as in the standard variety.

When comparing the vigour of the twin progenies and the standard, the different weighings were observed to give rather different results. This implies that in the beginning, when the plants were young, the twin progenies were more vigorous in relation to the standard than

later on. Thus, at the first weighing in 1937 the total average of the twin progenies was $140{,}25 \pm 1{,}70$ gr., the corresponding average of the standard, $123{,}15 \pm 4{,}64$ gr., being significantly lower.

However, in the following year, 1938, when the plants had wintered, the twin progenies yielded less than the standard, and this decrease continued also in 1939. If the standard average every time is given the value 100, the corresponding average of the twin progenies was 114 in 1937, 97 in 1938 and 91 in 1939. These values are based on the total yield, the value 97 in 1938 representing the sum of the yield in 1937 and 1938 and the value 91 in 1939 the sum of the weight in all three years. This obvious change in the relation between twin progenies and standard is probably due in the first place to a difference in winter hardiness. Further, the high weight of the twin progenies in the first summer may be connected with the fact that the seedlings of this material get a better start than the seedlings of the standard. Though germinating simultaneously with the standard, the seedlings of the twin progenies already at an early stage were conspicuously larger than the standard seedlings. This early difference is probably in part connected with the observed difference in seed size, the bigger seeds giving more vigorous seedlings. Thus, the higher chromosome number of the triploid twins is probably in this way advantageous to the early development of the offspring, and this initial advantage may partly be responsible for the good yield of the first crop. The good development of the offspring in the first year must also and perhaps in the first place be ascribed to a general effect of the increased chromosome number. Later on, however, the favourable relation between the yield of the twin progenies and the standard is disturbed by the insufficient average hardiness of the former.

4. CORRELATION BETWEEN CHROMOSOME NUMBER AND VIGOUR.

As the twin progenies consisted of plants with at least nine different chromosome numbers, it was desirable to test the possible occurrence of a correlation between chromosome number and vigour. For this study 184 individuals with known chromosome numbers were available. Considering first the correlation between chromsome number and total yield in the three years, the following average values were obtained in the different chromosome classes:

Chromosome number:	56	57	58	59	60	61	62	63	64
Average weight:	894	1013	1005	848	825	889	806	845	1050
Plant number:	13	12	25	39	38	26	8	15	8

These values do not reveal any obvious correlation. If, however, the material is concentrated to the three chromosome classes 56—58, 59—61 and 62—64 the following distribution of the variates is obtained (Table 4).

According to this table the plants having 59—61 chromosomes are probably less vigorous than those having 56—58 chromosomes, the average weight values being $849{,}8 \pm 28{,}4$ and $978{,}0 \pm 49{,}0$ respectively. The difference is $128{,}2 \pm \pm 56{,}6$ and D/m = 2,27. The plants with the highest chromosome numbers, 62—64, also seem to be more vigorous than those having intermediate numbers, but this difference is too slight to be of any significance.

The real occurrence of a correlation between chromosome number and vigour is strengthened by the following data. It was observed that at the last weighing in 1939 the weight differences between the chromosome classes were much more pronounced than at the first weighing in 1937.

In the first weighing the absolute weight values in the three chromosome classes were 184,0, 168,7 and 175,0 respectively. In the fourth weighing the corresponding values were found to be 266,0, 212,9 and 234,7. In the latter series the difference between the average values 266,0 and 212,0 was found to be $53{,}1 \pm 20{,}2$. This gives a D/m value of 2,63, which strongly indicates that the intermediate chromosome class in this year was less vigorous than the 56—58 class. For comparison it may also be convenient to give the relative values in the following manner:

	Chromosome classes		
	56—58	59—61	62—64
1st weighing:	109	100	104
4th » :	125	100	110

Evidently the weight minimum in the intermediate chromosome class is much more pronounced at the fourth weighing in 1939 than

TABLE 4. *Correlation between chromosome number and vigour in the offspring of triploid Phleum pratense.*

Chromosome number	Plant weight 0	150	300	450	600	750	900	1050	1200	1350	1500	1650	1800 gr.	n	M ± m
56—58			1	2	3	8	7	8	10	3	3	4	1	50	$978{,}0 \pm 49{,}0$
59—61	2	—	5	13	21	17	16	16	16	11	2			103	$849{,}8 \pm 28{,}4$
62—64		1	4	—	7	4	4	6	2	1	2			31	$888{,}0 \pm 63{,}3$

at the first weighing in 1937. This strongly indicates that the plants having 59—61 chromosomes are less resistant to frost and other adverse environmental conditions than those having exactly or approximately the euploid number 56. The plants with $\pm$ 63 chromosomes also seem to be better than the intermediate class, but this difference is not certain.

More evidence as to the occurrence of correlation between chromosome number and vigour was obtained from a comparison between the chromosome numbers of the plants taken at random and those selected on account of their high weight.

According to Table 2 the average chromosome number of the plants taken at random is approximately the same as the average chromosome number of the selected vigorous plants, the two values being 59,73 and 59,50 respectively. However, the two series seem to differ from each other in another respect, viz. the degree of variance. Among the selected plants the numbers approaching the extreme values 56 and 64 are relatively more numerous than those having intermediate chromosome numbers. This implies an increased variability in comparison with the other series, in which the variates are more concentrated in the middle. The variance values of the two series were found to be 5,45 and 3,87 respectively (Table 2). It is rather probable that the former variance is really greater than the latter, since the relation is $5{,}45 : 3{,}87 = 1{,}41$ and P about 0,1 (according to the tables by FISHER and YATES, 1938). This result represents additional evidence that the plants having the exact or approximate 8x constitution are slightly more vigorous than those having numbers intermediate between 56 and 63. It is also possible that the plants having $\pm$ 63 chromosomes are more vigorous than the intermediate class, but the evidence for this conclusion is less convincing.

5. FERTILITY.

As in the mother plants, fertility proved to be perfectly good in the twin progenies. Since, in order to get their weight, the field plants were cut down before the seeds were ripe, an estimation of seed setting could only be made on pot plants cultivated in the greenhouse. The plants, representing the whole range of chromosome numbers, had been taken from the field in order to raise progeny after isolation or crossing. In plants having flowered in crossing groups the number of seeds per cm. of the culm was found to range from 0 to 110, the average value being 55,7. This is a degree of seed setting quite comparable to that found in ordinary timothy (cf. MÜNTZING, 1935, p. 105).

More precise information as to fertility was gathered from an examination of pollen samples, which could be taken before the plants were cut and weighed. The following results were obtained:

	Per cent good pollen						
	75—80	— 85	— 90	— 95	— 100	n	M
Plants in the twin progenies: ..	1	—	5	39	167	212	96,25
Standard plants:		1	1	3	3	8	92,50

Besides the standard plants having 80—100 per cent good pollen there was one male sterile standard individual with non-dehiscing anthers.

According to these data pollen fertility in the twin progenies is perfectly good and at least as good as in the standard variety. The pollen samples in the twin progenies were in the first place taken from the plants with known chromosome numbers. As practically all the plants were found to have 90—100 per cent good pollen, there is no correlation between pollen fertility and chromosome number. The pollen is good irrespective of the chromosome number.

IV. DISCUSSION.

The twin plants with $2n = 63$ are undoubtedly autopolyploid. They develop from the same seeds as the sister twins with $2n = 42$ and are most probably the result of a union between unreduced ovules and reduced male gametes (MÜNTZING, 1937 a, p. 222). In a previous paper (MÜNTZING, 1935) the genomatic constitution of *Phleum pratense* was given as *NN AA BB*. Consequently the twins with 63 chromosomes should have the formula *NNN AAA BBB*.

It is true that among the three sets of 21 chromosomes, constituting the triploid twins, there are certainly gene differences, *Phleum pratense* being a cross-fertilizing species. However, just as the two sets of 21 chromosomes in diploid *pratense* conjugate quite normally in spite of the presence of gene differences, the same good conjugation should be expected in the triploid twins between the three sets of 21 chromosomes. This would give rise to a considerable frequency of trivalents of the constitution *NNN, AAA* and *BBB*, a maximum of 21 trivalents being possible. Obviously, however, this mode of conjugation was not met with, the trivalents being quite rare and no possibly occurring larger associations being observed with certainty. At diakinesis the number of trivalents was certainly not higher than one or two, and at first

metaphase the number was evidently still lower. Only in a few I—M groups could quite clear trivalents be distinguished, and in comparison with the bivalents they were quite exceptional.

The very low frequency of trivalents must evidently be considered in connection with the unexpectedly high frequency of bivalents. In the absence of trivalents, 28 was the most frequent number of bivalents, and if the cell contained one or a few trivalents, the sum of the bivalents and trivalents was not lower than 28 [1]. The correctness of these observations was verified by chromosome counts in the offspring. Of 186 plants examined not a single one had less than 56 chromosomes.

The increased number of bivalents must be due to intergenomatic pairing between the *N*, *A* and *B* genomes. Since the expected number of associations is increased by seven, from 21 to 28, two of the three genomes may be supposed to be homologous. Assuming arbitrarily that these genomes are *A* and *B*, their homology is better indicated by using the symbols A_1 and A_2. Thus, the genome formula of diploid and triploid *pratense* would be $NN\,A_1A_1\,A_2A_2$ and $NNN\,A_1A_1A_1\,A_2A_2A_2$ respectively. In the triploid the 28 bivalents formed would consist of $NN + A_1A_1 + A_2A_2 + A_1A_2$. The remaining seven *N* chromosomes evidently may sometimes associate with homologous chromosomes (probably in the first place with the other *N* chromosomes, cf. below p. 488), giving a few trivalents, but most of them appear as univalents. In a few cases the number of bivalents or bivalents + trivalents was found to be higher than 28. This can only be explained by assuming a certain extent of intragenomatic homology. Pending more accurate data on the occurrence of more than 28 associations at diakinesis and I—M in the triploid twins, a detailed consideration of this probable intragenomatic pairing may be postponed till later on. The important thing to be discussed now is the intergenomatic pairing and the very low frequency of trivalents.

The homology between at least two of the *pratense* genomes has already been demonstrated by NORDENSKIÖLD (1937). From crosses between *Phleum pratense* ($2n = 42$) and *P. nodosum* ($2n = 14$) this author obtained two hybrid plants having $2n = 28$. In these hybrids meiosis was found to be quite regular, 14_{II} being the typical I—M configuration. In a few cells a ring-shaped quadrivalent was also observed. The regular meiosis in this hybrid combination is evidently in perfect agreement with the mode of meiosis in the triploid *pratense* twins. Using

[1] With one possible exception (p. 473).

the same genome symbols, *Phleum nodosum* will have the constitution *NN*, *P. pratense* *NN* A_1A_1 A_2A_2 and the F_1 hybrids *NN* A_1A_2. The formation of 14 bivalents in the hybrid is most probably due to conjugation of *N* with *N* and A_1 with A_2. Thus, the A_1 and A_2 genomes pair regularly just as in the triploid *pratense* twins.

Though the homology of A_1 and A_2 helps to explain the high number of bivalents in the triploid *pratense* twins, it leaves the main problem unsolved, viz. the remarkably low frequency of trivalents. This is not due to a general inability of the *Phleum pratense* and *nodosum* chromosomes to form multivalents. In the material previously studied by MÜNTZING (1935) and NORDENSKIÖLD (1937) associations of 3, 4 or even 5 chromosomes occurred in various hybrids or autopolyploid forms. This is not surprising since the chiasma frequency is, indeed, sufficiently high to permit the formation of such multivalents. In the triploid twins ($2n = 63$) the majority of the I—M bivalents were rings with a least 2 chiasmata. At diakinesis the chiasma number in the bivalents was found to range from 1 to 3 or even 4 (Figs. 6—8). It also seems clear that the chiasmata are not localized to special regions, which would have been an obstacle to multivalent formation.

Thus, *the Phleum chromosomes are not incapable of forming multivalents, but there is a tendency for such associations to be formed only when there is no opportunity of pairing two-by-two.* In autotriploid *Phleum nodosum* ($2n = 21$) trivalents were not rare (NORDENSKIÖLD, 1937) one metaphase group reproduced showing the configuration $4_{III} + 3_{II} + 3_{I}$ (l. c. Fig. 5). In the triploid *Phleum pratense* ($2n = 63$) there are also three quite homologous *N* genomes besides the A_1 and A_2 genomes. Since the latter form only bivalents (A_1—A_1, A_2—A_2 and A_1—A_2) the pairing of the *N* genomes in the triploid *pratense* should be expected to be of exactly the same kind as in the triploid *nodosum*. This is not the case, however, the frequency of trivalents evidently being much lower in triploid *pratense* than in triploid *nodosum*. This difference may be attributed to the difference in absolute chromosome number. In triploid *nodosum*, having only 21 chromosomes, the chance that the three homologous chromosomes find each other and get paired at zygotene is much greater than in triploid *pratense*, in which the chromosome number is three times as high.

In this connection the results of UPCOTT (1939 a) are interesting. The chiasma frequency of the duplex tetraploid hybrid between *Primula floribunda* and *P. verticillata* was found to be slightly lower than in the parent species. Other tetraploids, equally allo and auto, were found to

obey the same reduction rule [1]. According to UPCOTT the most probable explanation is that in the tetraploids the more numerous chromosomes take longer to pair and therefore partially fail to associate, the frequency of chiasmata being proportional to the amount of pairing.

In the pentaploid *Phleum nodosum* × *pratense* hybrids ($2n = 35$), studied by NORDENSKIÖLD (1937), the preliminary data demonstrate a rather high degree of multivalent formation. Two metaphase groups reproduced represent the configurations $2_{IV} + 2_{III} + 8_{II} + 5_I$ and $2_V + 2_{III} + 8_{II} + 3_I$ (l. c. Figs. 3—4). Hybrids of this kind have the constitution $NNNA_1A_2$. If chromosome pairing in such hybrids proceeded along the same lines as in triploid *pratense*, the typical configuration should be $14_{II} + 7_I$. A low frequency of trivalents might also be expected, the sum of bi- and trivalents not being higher than 14. Evidently, the degree of multivalent formation is greater than expected, and this may perhaps be attributed to the difference in absolute chromosome number, 35 versus 63. — In the pentaploid hybrid not only the homology between the *N* genomes and the homology between A_1 and A_2 results in pairing but also a partial homology between the *N* and the *A* genomes. In the tetraploid *pratense* × *nodosum* hybrid discussed above a few quadrivalents were also observed. These must also result from the occasional association of *A* and *N* chromosomes.

It is remarkable that in triploid *pratense* pairing between the A_1 and A_2 genomes is much more easily realized than pairing between the three A_1 genomes or the three A_2 genomes. Thus, bivalents of the type A_1—A_2 are regularly formed in spite of the fact that the A_1 and A_2 genomes might be supposed to be only partially homologous. On the other hand, the perfectly homologous associations A_1—A_1—A_1 and A_2—A_2—A_2 are very seldom realized. If the low frequency of multivalents in the plants with $2n = 63$ was due solely to the high absolute chromosome number, making it difficult for the homologous chromosomes to find each other, it is hard to understand why the seven A_1—A_2 bivalents are regularly formed and not A_1—A_1—A_1 and A_2—A_2—A_2 trivalents.

The possibility is not excluded that the A_1 and A_2 genomes are in reality perfectly homologous (cf. below p. 493), but even on this assumption the mode of chromosome conjugation in triploid *pratense* is peculiar. Moreover, if A_1 and A_2 are homologous they should be expected to give

[1] This rule, however, is not without exceptions. In autotetraploid *Dactylis glomerata* the chiasma frequency was the same as or even somewhat higher than in diploid *Dactylis Aschersoniana* (MÜNTZING, 1937 b, p. 154).

multivalents in pure *Phleum pratense*, but apart from rare exceptions such multivalents are not formed, the typical I—M configuration of *Phleum pratense* being 21_{II}.

As already pointed out above, the only possibility is to assume that the tendency to two-by-two pairing in this species is much more marked than the tendency to an association of three or more homologous chromosomes.

In *Pyrus malus* the number of bivalents is also higher in triploids than in diploids (NEBEL, DARLINGTON and MOFFETT, cited from DARLINGTON, 1937, p. 207). However, triploid *Pyrus malus* differs from the corresponding form of *Phleum pratense* by showing a rather high frequency of trivalents, and in addition there are also larger associations.

The cytological conditions in *Phleum* are paralleled to a large extent by similar phenomena in *Solanum nigrum* (JØRGENSEN, 1928). This is a polyploid species, the chromosome number being $2n = 72$. Though irregularities were not entirely absent, meiosis in the diploid *nigrum* was generally characterized by an almost mechanical regularity. The first and second metaphase plates were always found to contain 36 chromosomes. At diakinesis there were probably 36 gemini, though the presence of a few trivalents or quadrivalents was not quite excluded. In haploid *nigrum* 36 univalents might be expected at meiosis, but the homology between the genomes constituting the species revealed itself by the formation of $12_{II} + 12_{I}$ in typical cases. No quite clear associations of three chromosomes were observed in the p. m. c., but in the megaspore mother cells a few trivalents could be distinguished. This indicates that *S. nigrum* contains three more or less homologous genomes (JØRGENSEN, l. c. p. 195).

The meiotic conditions in triploid *Solanum nigrum* are of still more interest with regard to our material. According to JØRGENSEN (l. c. pp. 181—183), most of the 108 chromosomes form bivalents, the number of elements present at I—M varying between 50 and 65. Since only bivalents and univalents seemed to occur, it was concluded that the three sets of 36 chromosomes combined in such a way that two of them formed 36_{II}, the chromosomes of the third set pairing inter se. Thus, the triploid *nigrum* behaved as if composed of a diploid and a haploid, the scheme $36_{II} + 12_{II} + 12_{I}$ being approached.

Evidently, the mode of meiosis in this triploid is of exactly the same type as in *Phleum pratense*. In both species trivalents are absent or at least rather rare. This is very striking, since the perfect homology

between the three sets of chromosomes would be expected to permit the union of all the chromosomes into trivalents. Thus, in these triploids other strong factors besides chromosome homology are of prime importance for the mode of chromosome association.

In other respects there are some differences between the triploids of the two species. The *Phleum* triploid is perfectly fertile, and in the progeny the chromosome number is never lower than 56. This is due to a general regularity of meiosis and a regular formation of tetrads. The triploid *Solanum nigrum*, on the contrary, was rather sterile, and at the tetrad stage from two to eight cells were formed. JØRGENSEN (l. c. p. 183) also mentions that according to WINKLER's breeding experiments (1922) the offspring from the triploids in a few generations turn into diploids.

Since the triploids in *Phleum pratense* and *Solanum nigrum* are both characterized by rather high chromosome numbers, it is of interest to compare these cases with other triploids having high chromosome numbers. Such material is, for instance, represented by triploid *Nicotiana Tabacum*, having $2n = 72$. Triploids of this kind have been examined by GOODSPEED (1930) and EAST (1933). GOODSPEED observed trivalents as well as bivalents and univalents at I—M, but the exact number could not be determined. Judging from the total number of elements at I—M, which was frequently higher than 36, the average number of trivalents was probably lower than 12. In his triploid EAST (l. c.) found that the number of chromosomal bodies at I—M varied from 36 to 42, the former number being most typical. Though a few of these bodies may correspond to univalents or trivalents, most of them are considered to be bivalents. If this is true, meiosis in triploid *Nicotiana Tabacum* would be rather similar to meiosis in triploid *Phleum pratense*. At any rate, the frequency of trivalents seems to be relatively low and far lower than the possible maximum of 24. — Meiosis has also been studied in autotriploid twin plants of *Triticum vulgare*, having $2n = 63$ just as our *Phleum* twins (YAMAMOTO, 1936). In this material, in contrast to *Phleum*, *Solanum* and *Nicotiana*, the frequency of trivalents seems to be rather high, but no exact counts of the number of trivalents were made.

In comparison with our observations in *Phleum* the cytogenetic results obtained by SHIMOTOMAI (1931, 1932, 1933) in hybridization experiments in *Chrysanthemum* are quite interesting. The meiotic behaviour in the *Chrysanthemum* hybrids studied seems to depend entirely on the chromosome number of the parents. If the difference in gametic

chromosome number between the species crossed is an even multiple of the basic chromosome number 9 (generally 18), the resulting hybrid will have perfectly regular meiosis with bivalents only. If the numerical difference between the parents is an uneven multiple (generally 9), the supernumerary chromosomes cannot find any mates and as a rule appear as univalents at meiosis. A cross 18×36 will consequently give a hybrid with $2n = 54$, which chromosomes form 27 bivalents at meiosis. A cross of the type 18×27, on the contrary, gives an F_1 with $18_{II} + 9_I$ at meiosis. In hybrids of the first kind all gametes receive the same chromosome number, and such hybrids therefore behave as new cytologically constant species in contrast to hybrids of the second kind. In the *Chrysanthemum* material studied the different genomes, judging from the mode of pairing, must be largely homologous and may be recombined at will without serious disturbances. The behaviour of the hybrids seem to depend on purely numerical conditions. These results are quite similar to those found e. g. in *Papaver* (LJUNGDAHL, 1924) and in *Fragaria* (LILIENFELD, 1933). For other similar cases cf. DARLINGTON, 1937, p. 208.

Thus, though the genomes of the polyploid *Chrysanthemum* species in question are largely homologous, there are no multivalents at meiosis in the pure species or in balanced hybrids derived from them. In hybrids with univalents, however, trivalents may also occur. In the *Chrysanthemum* hybrid *lavandulaefolium* × *indicum* the frequency of trivalents was even found to be rather high (TAKEMOTO, 1939), configurations such as $8_{III} + 1_{II} + 1_I$ and $5_{III} + 4_{II} + 4_I$ being observed. Of the two parent species, *C. lavandulaefolium* is diploid ($2n = 18$) and *C. indicum* tetraploid ($2n = 36$). Though the two genomes of *indicum* must be largely homologous, which was also verified by chromosome morphological studies (SHIMOTOMAI and TAKEMOTO, 1939), they do not seem to form quadrivalents in pure *C. indicum* (cf. SHIMOTOMAI, 1933, Fig. 1 g).

The absence of multivalents in the pure species or balanced hybrids seemed peculiar at a previous discussion of the *Chrysanthemum* results (MÜNTZING, 1936), but in view of the present results in *Phleum* it is more easy to understand. It is not necessary to assume a condition of differential affinity, the absence of multivalents may simply be the result of a strong tendency to two-by-two pairing of the same kind as in *Phleum*. If this tendency is at work, the »need» of association is satisfied by the pairing of two homologous chromosomes. Even if there are

groups of four perfectly homologous chromosomes, these will pair as two bivalents and not as one quadrivalent.

In *Solanum nigrum* not only haploids and triploids were studied by JØRGENSEN (1928) but also tetraploids. Considering the remarkably regular I—M association in the triploid, it is not surprising that in the tetraploid ($2n = 144$) multiple associations were quite rare, the great majority of the chromosomes forming bivalents. Only a few quadrivalents were observed at diakinesis and may also occur at first metaphase.

In *Phleum pratense* tetraploid forms ($2n = 84$) have not yet been produced, but their production is probably only a question of time. They might either be produced by colchicine treatment of ordinary timothy or by selection of twin seedlings in the progeny of 8x plants. As described in detail in this paper, many plants having exactly or approximately the 8x chromosome number 56 were obtained in the offspring of the triploid twins. By raising new twin seedlings from 56-chromosome plants it should be relatively easy to get plants with $56 + 28 = 84$ chromosomes. These would be tetraploid in relation to ordinary *Phleum pratense* ($2n = 42$). By crosses between plants with 84 and 56 chromosomes it should also be possible to raise individuals with $2n = 70$. In view of the strong tendency to two-by-two pairing in *Phleum*, it may be predicted that such plants should in the main have a regular meiosis with 35 bivalents. In the same way the tetraploid individuals ($n = 84$) should mainly have 42 bivalents at diakinesis and first metaphase. — Starting again from plants with 70 and 84 chromosomes, it should be possible to produce true breeding strains with still higher chromosome multiples.

Pending the production of such new strains, the mode of chromosome association in ordinary *Phleum pratense* may be considered once more from another point of view. As observed by MÜNTZING (1935) and as described in the present paper, the typical I—M configuration is 21_{II}. The absence or rarity of multivalents would seem to indicate that the three genomes of *Phleum pratense* are well differentiated. However, the results of NORDENSKIÖLD (1937) and ourselves strongly indicate that at least two of the three genomes (A_1 and A_2) are highly homologous. This was evident, firstly by the regular formation of 14 bivalents in the hybrid between *Phleum pratense* and *nodosum* and, secondly, by the formation of 28 bivalents (+ 7 univalents) in our triploid *pratense*. It should also be observed that the tetraploid *pratense* × *nodosum* hybrid ($2n = 28$) as well as triploid *pratense* ($2n = 63$) have

very good fertility, and that in the offspring of triploid *pratense* variation in plant vigour was not greater than in ordinary *Phleum pratense*.

The absence of quadrivalents in *P. pratense* does not necessarily imply that the bivalents formed are always of the type A_1—A_1 and A_2—A_2. It may be that pairing is just as frequent between the A_1 and A_2 chromosomes. Among the four homologous genomes present the pairing tendency is completely satisfied by the formation of bivalents. It is quite possible that these bivalents are formed at random, and if that is true, the distinction between A_1 and A_2 is no longer valid. Thus, it seems possible and even probable that the genome formula of *P. pratense* should be given as *NN AA AA* instead of *NN* A_1A_1 A_2A_2.

Studies of segregation ratios in *Phleum pratense* would be important in order to decide if the above hypothesis of random pairing is correct. If there is no distinction between A_1 and A_2 genomes, dihybrid ratios for factors located in these genomes would be of the autotetraploid type 35 : 1 rather than 15 : 1. Unfortunately, as far as we know, distinct segregation ratios have not yet been described for this species.

Such ratios, however, are known in *Solanum tuberosum*, in which the cytogenetic conditions seem to be similar to those in *Phleum pratense*. LUNDEN (1937) studying the genetics of the species found autotetraploid segregation ratios for several factors. This is remarkable since *S. tuberosum* has generally been assumed to be an allotetraploid species. All European potato varieties have $2n = 48$, and at meiosis the great majority of these chromosomes form bivalents, only a small number forming true multiple associations (BLEIER, 1931; MEURMAN and RANCKEN, 1932; ELLISON, 1936). Under such circumstances it must be concluded that in the potato the four genomes of 12 chromosomes are highly homologous, and that there is a random two-by-two pairing between the homologous chromosomes of all four genomes. *Solanum tuberosum* like *Phleum pratense* may, indeed, be one of those species in which four homologous chromosomes prefer to associate at random as two pairs instead of forming a quadrivalent.

This possibility is further strengthened by the cytological observations of LAMM (1938) and PROPACH (1937, 1938). LAMM studied a polyhaploid potato plant ($2n = 24$), being a member of a pair of twin seedlings in the progeny of a tetraploid F_1 hybrid ($2n = 48$) between triploid *Solanum chaucha* (from South America, $2n = 36$) and tetraploid *S. tuberosum* ($2n = 48$). In this haploid the 24 chromosomes were associated at I—M as 12 bivalents in the majority of the p. m. c., and many of these bivalents were joined by two chiasmata. In the

tetraploid sister twin most of the chromosomes as usual formed bivalents, though some trivalents and quadrivalents were also present. The average chiasma frequency per chromosome was found to be 1,22 in the haploid, 1,27 in the tetraploid.

Thus, it seems clear that *Solanum tuberosum* belongs to the same group of material as *Solanum nigrum, Phleum pratense,* some *Chrysanthemum* and *Papaver* species, etc., for which a high degree of autopolyploidy is characteristic though this is not apparent at meiosis in the pure species.

The number of *Solanum* species showing such conditions are not limited to *S. nigrum* and *tuberosum.* PROPACH (l. c.) has studied some other polyploid *Solanum* species, showing quite regular bivalent formation in the pure species but autosyndesis in hybrids with diploid species. PROPACH (1937) concludes that the supposed allopolyploidy of *S. acaule* and *demissum* is only apparent, their genomes in reality being homologous. Though autopolyploid the species are supposed to have acquired, during their phylogeny, the capacity of regular bivalent formation.

According to our opinion, and in agreement with PROPACH's conclusions, *the results in Phleum and the other genera, showing similar conditions, can only be explained by assuming a special genotypically controlled tendency to bivalent formation. In species capable of such a control the formation of multivalents is prevented, and thus a regular meiosis is secured in spite of complete or almost complete homology between more than two genomes.* — By assuming such a force we do not deny the prime importance of differential affinity and chiasma frequency for the mode of chromosome pairing (cf. DARLINGTON, 1937), but these factors do not seem to be sufficient to explain the whole story. — In presumably autotetraploid *Tulipa* species UPCOTT (1939 b) finds a low frequency of quadrivalents, and this is correlated with a lower chiasma frequency and fewer changes of partner at pachytene than in diploid and triploid tulips. Judging from these observations, the specific genes preventing multivalent formation may excersize their influence either by a reduction in the frequency of chiasmata in the regions paired or by a reduction of the changes of partner at pachytene. The latter method may be the more important one for the cases discussed in this paper. In *Phleum,* at least, the chiasma frequency was high enough to permit the formation of multivalents.

From the facts described above it is clear that some species, though having a regular meiosis without multivalents, are nevertheless highly

autopolyploid. Thus, though *the presence of multivalents at meiosis may indicate autopolyploidy, the absence of multivalents does not prove the species to be allopolyploid.* In a paper on the evolutionary significance of autopolyploidy one of us (MÜNTZING, 1936) presented evidence strongly indicating that the rôle of autopolyploidy in nature had been underestimated. This was especially evident from an analysis of the properties of a series of intraspecific chromosome races and closely related species representing different degrees of polyploidy.

In most of the cases studied the polyploid forms were characterized by the presence of multivalents at meiosis, but in a minority of cases there were no multivalents in the polyploid forms. In some of the latter cases (*Nasturtium*, *Chrysanthemum* and *Betula*) other evidence than the presence of multivalents strongly indicated autopolyploidy, and therefore the conclusion was drawn (l. c. p. 313) that »though a few cases occur, in which the absence of multivalents indicates allopolyploidy, these are only exceptions and in some cases even uncertain exceptions to the rule that polyploid intraspecific chromosome races are generally autopolyploid».

This conclusion is further supported by the evidence discussed in the present paper, firstly, because it is now quite clear that genomatic homology does not always lead to multivalent formation, secondly, because two of the apparent exceptions to the rule were represented by *Phleum alpinum* and *Phleum pratense-nodosum*. Considering the *Phleum* results presented in this paper and those obtained by NORDENSKIÖLD (1937), it is quite evident that the *Phleum* species in question contain genomes that are completely or partially homologous. Thus, the exceptions to the rule that polyploid intraspecific chromosome races are autopolyploid now seem to be still fewer than in 1936.

It is also evident that the occurrence of autopolyploidy is not limited to polyploid chromosome races but is also met with in polyploid species. So far only such species as are characterized by multivalents at meiosis have been recognized with certainty as being autopolyploid (cf. MÜNTZING, 1936, p. 334). However, the examples in the genus *Solanum*, for instance, demonstrate that this category also includes species with only bivalents at meiosis. Thus, *races and species, which are completely or predominantly autopolyploid, must be even more frequent than there was reason to assume a few years ago* [1].

[1] In her recent paper on polyploidy in *Tulipa*, UPCOTT (1939 b) finds it appropriate to critisize the paper of MÜNTZING (1936) by confronting two detached and apparently contradictory sentences. However, anyone reading the whole chapter will

Though, from a theoretical point of view, the mode of chromosome pairing is of most interest in our triploid *Phleum* twins, some other points may also be briefly considered. — It is rather striking that in the progeny of the twins with $2n = 63$ the plants are but very slightly sensitive to the variation in chromosome number, plants with 59—61 chromosomes having about the same vigour as plants with 56—58 and 62—64 chromosomes. This independence of chromosome number is certainly due to the autopolyploid constitution and the rather high absolute chromosome number of the material. Quite analogous results were obtained by MÜNTZING (1937 b, 1940) in material of *Dactylis* and *Poa*. Also in allopolyploid *Triticale* strains deviations from the typical chromosome number 56 have not much effect on plant vigour (MÜNTZING, 1939).

The high degree of fertility in the *Phleum* material under discussion may be explained in the same way. All genes necessary for the functioning of the pollen grains and ovules are reduplicated, and therefore it does not matter whether there are any extra chromosomes present or not.

From a practical point of view this undisturbed fertility is of course very favourable, and the same is true of the regular meiosis in the triploid twins. Since there is every reason to believe that the same regularity will be repeated in the offspring, it should be an easy task to raise an unlimited number of stable strains, having $2n = 56$. Whether the other possible derivatives, having $2n = 70$ and $2n = 84$ etc., will be quite stable is so far an open question. — For breeding purposes it

---

not misunderstand the meaning. Since probably nothing can be said against the first statement, that the presence of multivalents indicates autopolyploidy, the absence of multivalents allopolyploidy, the other sentence critized may be considered, viz. »Thus, though a few cases occur, in which the absence of multivalents indicates allopolyploidy, these are only exceptions and in some cases even uncertain exceptions to the rule that polyploid intraspecific chromosome races are generally autopolyploid.»

Since the evidence on which this statement is based is still perfectly valid, there is no reason for a change of opinion. On the contrary, the data discussed above in the present paper give further support to the view that polyploid intraspecific chromosome races are really in most cases autopolyploid.

In the paper by UPCOTT (l. c. p. 335) it is further stated, strangely enough in contradistinction to some quotations from the paper by MÜNTZING (1936), that many polyploid species are intermediate between the two extremes (presumably auto- and allopolyploidy). We perfectly agree with this opinion, especially since chapter VII in MÜNTZING's paper (l. c. pp. 361—365) deals with the same subject and reaches the same conclusions.

would be of most interest to concentrate on the production of strains with 56 chromosomes. This program is further supported by the observation of a very slight, but nevertheless significant, correlation between chromosome number and vigour, the plants having the exact or approximate chromosome number 56 being somewhat superior to individuals with chromosome numbers intermediate between 56 and 63.

In spite of the presence of individuals of the latter kind some of the twin progenies studied gave a yield which was quite or almost as good as the yield of the standard ($2n = 42$). This is remarkable, since the standard used was a well-known commercial variety and the first triploid twins available were of unknown origin. Good future results may be expected a) by the production of 63-chromosome plants from the best *P. pratense* material available, b) by selection and intercrossing among the triploid twins thus produced, and c) by selection of strains with 56 chromosomes in the following generations. — Due to meiotic elimination the chromosome number in the offspring of 63-chromosome twins will in time probably automatically reach the 8x condition, but by a direct selection of vigorous plants with exactly or approximately 56 chromosomes the process may be greatly accelerated.

Finally, it may be mentioned that the change in chromosome number from 42 to 56—64 does not involve any obvious change in the chemical properties. Thanks to Dr. J. LINDBERG, Svalöf, determinations of water content, crude protein, crude fat, soluble carbohydrates, crude fibre and ashes were undertaken in the twin pairs, twin progenies and the standard variety. In all of these properties the material with high chromosome numbers had sometimes slightly higher, sometimes slightly lower values than the corresponding types with the normal chromosome number. Thus, the breeding of timothy with 56 chromosomes instead of 42 may evidently be undertaken without the risk of a decreasing chemical quality.

SUMMARY.

1. Twin plants of *Phleum pratense*, having $2n = 63$ instead of the normal number $2n = 42$, were found to have good vigour and perfectly good fertility. The »triploid» members of the twin pairs have longer, broader and thicker leaves than the corresponding diploids and also thicker stems, longer and thicker culms, bigger spikelets and larger pollen grains.

2. Meiosis was studied in the p. m. c. In the triploids the fre-

quency of bivalents was much higher and the frequency of trivalents much lower than expected. The sum of the bivalents and the occasional trivalents was hardly ever lower than 28, and the number of univalents not higher than seven.

3. Estimating the degree of meiotic elimination the average chromosome number of the gametes formed by the triploids was found to be 30,03. All gametes will carry at least 28 chromosomes. In the offspring of the triploid twins chromosome counts were undertaken in 186 individuals. The chromosome number was found to range from 56 to 64, the average value being 59,66. Thus, the agreement between expectation and observation is quite good.

4. Plant weight was compared in the twin progenies and a standard variety having the normal chromosome number 42. Considering the chromosomal variation in the twin progenies, their average productivity was surprisingly good and about equal to that of the standard. In the first summer the twin progenies gave a higher yield than the standard, but in the later weighings the results were less favourable. Thus, the twin progenies were less hardy than the standard.

5. In the twin progenies the possible occurrence of a correlation between chromosome number and vigour was tested. Combining all data available, the plants having the exact or approximate 8x constitution were found to be slightly more vigorous than those having numbers intermediate between 56 and 63. Pollen fertility, on the other hand, was found to be quite good in plants with any chromosome number.

6. The mode of chromosome pairing in diploid and triploid *Phleum pratense* is discussed. Two of the three genomes of the species must be homologous, and thus the genome formula should be given as *NN* A_1A_1 A_2A_2 rather than *NN AA BB*. It is even possible that A_1 and A_2 are identical, and that pairing between the A_1 and A_2 chromosomes is just as frequent as the pairing of the type A_1—A_1 and A_2—A_2.

7. In *Phleum pratense* the »need» of association is almost completely satisfied by the pairing of two homologous chromosomes, even if plenty of other homologous chromosomes are present in the nucleus. This is not due to an insufficient chiasma frequency but must be caused by a special, genotypically controlled tendency to bivalent formation. Similar cases in other genera are discussed.

8. Since an autopolyploid constitution is not always accompanied by multivalent formation, races and species which are completely or

mainly autopolyploid must be more frequent than there was earlier reason to assume.

9. The mode of production and practical importance of timothy strains with 56, 70 and 84 chromosomes are discussed.

LITERATURE CITED.

1. BLEIER, H. 1931. Untersuchungen über die Sterilität der Kartoffel. — Archiv für Pflanzenbau, Bd. 5, H. 3, pp. 545—560.
2. DARLINGTON, C. D. 1937. Recent advances in cytology. 2nd Ed. — London.
3. EAST, E. M. 1933. The behaviour of a triploid in *Nicotiana Tabacum* L. — Amer. Journ. of Botany, Vol. 20, pp. 269—289.
4. ELLISON, W. 1936. Meiosis and fertility in certain British varieties of the cultivated potato (*Solanum tuberosum* L.). — Genetica XVIII, H. 3/4, pp. 217—254.
5. FISHER, R. A. and YATES, F. 1938. Statistical tables for biological, agricultural and medical research. — London.
6. GOODSPEED, T. H. 1930. Occurrence of triploid and tetraploid individuals in X-ray progenies of *Nicotiana Tabacum*. — Univ. Calif. Publ. Bot. Vol. 11, No. 17, pp. 299—308.
7. JØRGENSEN, C. A. 1928. The experimental formation of heteroploid plants in the genus *Solanum*. — Journ. of Genetics, Vol. XIV, No. 2, pp. 133—211.
8. LAMM, R. 1938. Notes on a haploid potato hybrid. — Hereditas XXIV, pp. 391—396.
9. LILIENFELD, F. A. 1933. Karyologische und genetische Studien an *Fragaria*. Ein tetraploider fertiler Bastard zwischen *F. nipponica* (n = 7) und *F. elatior* (n = 21). — Jap. Journ. of Botany, Vol. VI, No. 3, pp. 425—458.
10. LJUNGDAHL, H. 1924. Über die Herkunft der in der Meiosis konjugierenden Chromosomen bei *Papaver*-Hybriden. — Svensk Botan. Tidskrift, Bd. 18, pp. 279—291.
11. LUNDEN, A. P. 1937. Arvelighetsundersøkelser i potet (*Solanum tuberosum* L.). — Meldinger fra Norges Landbrukshøiskole, pp. 1—156.
12. MEURMAN, O. and RANCKEN, G. 1932. Untersuchungen über die Chromosomenverhältnisse bei kultivierten Kartoffelsorten (*Solanum tuberosum* L.). — Societas Scientiarum Fennica. Commentationes Biologicae III, 20.
13. MÜNTZING, A. 1935. Cytogenetic studies on hybrids between two *Phleum* species. — Hereditas XX, pp. 103—136.
14. — 1936. The evolutionary significance of autopolyploidy. — Hereditas XXI, pp. 263—378.
15. — 1937 a. Polyploidy from twin seedlings. — Cytologia, FUJII Jub. Vol., pp. 211—227.
16. — 1937 b. The effects of chromosomal variation in *Dactylis*. — Hereditas XXIII, pp. 113—235.
17. — 1938 a. Note on heteroploid twin plants from eleven genera. — Hereditas XXIV, pp. 487—491.

18. MÜNTZING, A. 1938 b. Resultat och erfarenheter från verksamheten vid Sveriges Utsädesförenings kromosomavdelning. — Lantbruksveckans Handlingar, pp. 1—11.

19. — 1939. Studies on the properties and the ways of production of rye-wheat amphidiploids. — Hereditas XXV, pp. 387—430.

20. — 1940. Further studies on apomixis and sexuality in *Poa*. — Hereditas XXVI, pp. 115—190.

21. NORDENSKIÖLD, H. 1937. Intra- and interspecific hybrids of *Phleum pratense* and *P. alpinum*. — Hereditas XXIII, pp. 304—316.

22. PROPACH, H. 1937. Cytogenetische Untersuchungen in der Gattung *Solanum*, Sect. *Tuberarium*. II. Triploide und tetraploide Artbastarde. — Zschr. f. ind. Abstamm.- u. Vererb.-Lehre, Bd. LXXIII, H. 1, pp. 143—154.

23. — 1938. Cytogenetische Untersuchungen in der Gattung *Solanum*, Sect. *Tuberarium*. IV. Tetraploide und sesquidiploide Artbastarde. — Zschr. f. ind. Abstamm.- u. Vererb.-Lehre, Bd. LXXIV, H. 3/4, pp. 375—387.

24. SHIMOTOMAI, N. 1931. Bastardierungsversuche bei *Chrysanthemum*. I. — Journ. of Science of the Hiroshima Univ., Series B. Div. 2, Vol. 1, pp. 37—54.

25. — 1932. Bastardierungsversuche bei *Chrysanthemum*. II. Entstehung eines fruchtbaren Bastardes (haploid 4n) aus der Kreuzung von *Ch. marginatum* (hapl. 5n) mit *Ch. morifolium* (hapl. 3n). — Journ. of Science of the Hiroshima Univ., Series B. Div. 2, Vol. 1, pp. 117—120.

26. — 1933. Zur Karyogenetik der Gattung *Chrysanthemum*. — Journ. of Science of the Hiroshima Univ., Series B. Div. 2, Vol. 2, pp. 1—100.

27. SHIMOTOMAI, N. und TAKEMOTO, T. 1939. Über die Morphologie der Chromosomen bei 6 Arten von *Chrysanthemum*. — Journ. of Science of the Hiroshima Univ., Series B. Div. 2, pp. 201—204.

28. SYLVÉN, N. 1929. Själv- och korsbefruktning hos timotej och hundäxing. — N. J. F:s kongress i Helsingfors, Juli 1929, Sektion 3, pp. 1—21.

29. TAKEMOTO, T. 1939. Über die Morphologie der Chromosomen bei einer Art und zwei Bastarden von *Chrysanthemum*. — Journ. of Science of the Hiroshima Univ., Series B, Div. 2.

30. UPCOTT, M. 1939 a. The nature of tetraploidy in *Primula kewensis*. — Journ. of Genetics, Vol. XXXIX, No. 1, pp. 79—100.

31. — 1939 b. The genetic structure of *Tulipa*. III. Meiosis in polyploids. — Journ. of Genetics, Vol. XXXVII, No. 2, pp. 303—339.

32. VALLE, O. 1931. Untersuchungen über die Selbststerilität und Selbstfertilität des Timothées (*Phleum pratense* L.). — Acta Agralia Fennica 24, pp. 1—258.

33. WINKLER, H. 1922. Über die Entstehung von genotypischer Verschiedenheit innerhalb einer reinen Linie. — Deutsche Ges. f. Vererb.-wiss., Versammlungsbericht, Zschr. f. ind. Abstamm.- u. Vererb.-Lehre, Bd. XXVII, pp. 244—245.

34. YAMAMOTO, Y. 1936. Über das Vorkommen von triploiden Pflanzen bei Mehrlingskeimlingen von *Triticum vulgare* VILL. — Cytologia 7, pp. 431—436.

35. ÅKERMAN, Å., GRANHALL, I., NILSSON-LEISSNER, G., MÜNTZING, A. and TEDIN, O. 1938. Swedish contributions to the development of plant breeding. — Stockholm (New Sweden Tercentenary Publications).

CONTENTS.

I. Introduction 463

II. The properties of the triploid twins 463

1. Morphology and fertility 463
2. Meiosis 468

III. Progenies of triploid twins 478

1. Material 478
2. Chromosomal variation 478
3. Plant weight 480
4. Correlation between chromosome number and vigour 482
5. Fertility 484

IV. Discussion 485

Summary 497

Literature cited 499